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Jeffrey [US/US]; 10301 Sorrel Avenue, Potomac, MD  
20854 (US).

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**(74) Agent:** BARTOSZEWICZ, Lola, A.; Sim & McBurney,  
330 University Avenue, 6th Floor, Toronto, Ontario M5G  
1R7 (CA).

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**(71) Applicants (for all designated States except US):**  
**AVENTIS PASTEUR LIMITED** [CA/CA]; Connaught Campus, 1755 Steeles Avenue West, Toronto, Ontario M2R 3T4 (CA). **THERION BIOLOGICS** [US/US]; 76 Rodgers Street, Cambridge, MA 02142 (US). **NATIONAL CANCER INSTITUTE** [US/US]; Executive Plaza South, Suite 450, 6120 Executive Blvd., Rockville, MD 20852 (US).

**(72) Inventors; and**

**(75) Inventors/Applicants (for US only):** **BERNSTEIN, Neil** [CA/CA]; 31 Burton Road, Toronto, Ontario M5P 1V1 (CA). **TARTAGLIA, James** [US/US]; 7 Christina Drive, Schenectady, NY 12303 (US). **TINE, John, A.** [US/US]; 215 Broad Street, Scotia, NY 12302 (US). **PANICALI, Dennis, L.** [US/US]; 76 Rogers Street, Cambridge, MA 02142 (US). **GRITZ, Linda** [US/US]; 76 Rogers Street, Cambridge, MA 02142 (US). **SCHLOM,**

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**WO 02/10379 A2**

**(54) Title:** MODIFIED CEA AND USES THEREOF

**(57) Abstract:** The invention discloses immunogenic CEA agonist polypeptides/proteins comprising a modified epitope containing the amino acid sequence YLSGADLNL, nucleic acids coding therefor, vectors and/or cells comprising said nucleic acids, and mixtures and/or compositions thereof. Methods for eliciting or inducing CEA-specific immune responses utilizing the aforementioned agents are also disclosed.

## Modified CEA and Uses Thereof

### **FIELD OF THE INVENTION**

The present invention relates to immunology, in particular to novel  
5 biologically active modified CEA agonist polypeptides/proteins containing a modified epitope therein, nucleic acids coding therefor, vectors and/or cells comprising said nucleic acid, mixtures and/or compositions of the aforementioned, and their use as immunogenic agents and/or in treatments of cancer.

### **10 BACKGROUND OF THE INVENTION**

Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. The disclosure of these references are hereby incorporated by reference into the present disclosure.

The prospects of cancer immunotherapy rely upon the identification of tumor  
15 associated antigens which can be recognized by the immune system. Specifically, target antigens eliciting T cell-mediated responses are of critical interest. This comes from evidence that cytotoxic T lymphocytes (CILs) can induce tumor regression both in animal models (Kast W. et al (1989) *Cell* 59:6035; Greengberg P. (1991) *Adv. Immunol.* 49:281) and in humans (Boon T. et al. (1994) *Annu. Rev. 20 Immunol.* 12:337).

Human carcinoembryonic antigen (CEA) is a 180 kD glycoprotein expressed on the majority of colon, rectal, stomach and pancreatic tumors (Muaro et al. (1985) *Cancer Res.* 45:5769), some 50% of breast carcinomas (Steward et al. (1974) *Cancer* 33:1246) and 70% of lung carcinomas (Vincent, R.G. and Chu, T.M. (1978) *J. Thor. Cardiovas. Surg.* 66:320). CEA is also expressed in fetal gut tissue and to a lesser extent on normal colon epithelium. The immunogenicity of CEA has been ambiguous, with several studies reporting the presence of anti-CEA antibodies in patients (Gold et al. (1973) *Nature New Biology* 239:60; Pompecki, R. (1980) *Eur. J. Cancer* 16:973; Ura et al. (1985) *Cancer Lett.* 25:283; Fuchs et al. (1988) *Cancer Immunol. Immunother.* 26:180) while other studies have not (LoGerfo et al. (1972) *Int. J. Cancer* 9:344; MacSween, J.M. (1975) *Int J. Cancer* 15:246; Chester K.A. and Begent, H.J. (1984) *Clin. Exp. Immunol.* 58:685). CEA was first described as a

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cancer specific fetal antigen in adenocarcinoma of the human digestive tract in 1965 (Gold, P. and Freeman, S.O. (1965) *Exp. Med.* 121:439). Since that time, CEA has been characterized as a cell surface antigen produced in excess in nearly all solid tumors of the human gastrointestinal tract. The gene for the human CEA protein has 5 been cloned (Oikawa et al (1987) *Biochim. Biophys. Res.* 142:511-518; European Application No. EP 0346710).

Notwithstanding the aforementioned ambiguous demonstration of CEA's immunogenicity, a phase I clinical trial using a vaccinia-CEA vaccine ("rV-CEA") 10 did demonstrate that CEA-specific cytolytic T-lymphocyte (CTL) response could be elicited in humans (Tsang, K.Y. et al. (1995) *J. Natl. Cancer Instit* 87:982-990; Tsang K.Y. et al. (1997) *Clin. Cancer Res.* 3:2439-2449). As a consequence of the studies, a CEA immunodominant CTL epitope was identified. This 9-mer (i.e. YLSGANLNL) was shown to bind to a HLA-A2 class I molecule and has been 15 designated carcinoembryonic antigen peptide-1 ("CAP-1"). Several subsequent studies have also demonstrated the ability of the CAP-I epitope/peptide *per se* to elicit CEA-specific human CTL responses (Alters, S.E. et al. (1998) *J. Immunother.* 21:17-26; Tsang, K.Y. et al. (1997) *Supra*; Zaremba, S. et al. (1997) *Cancer Res.* 57:4570-4577). Moreover, stable CTL lines derived by culture of peripheral blood 20 mononuclear cells (PBMCs) from rV-CEA vaccinated patients with CAP1 and interleukin (IL)-2 have recently been described (Tsang, K.Y. (1997) *Supra*).

It is an accepted principle that when an immunogenic peptide is modified in a conserved manner (i.e. a hydrophobic amino acid is substituted with a hydrophobic 25 amino acid) the modified peptide is likely to have similar immunogenic activity based upon the maintenance of the molecule's shape, charge and hydrophobic character. More specifically, a study by Madden (Madden et al. (1993) *Cell* 75:693) has identified specific amino acid preferences in peptides for MHC-complexing, a precursor step to T cell recognition. Madden as well as other investigators 30 (Rammensee et al., (1995) *Immunogenetics* 41:178) have suggested that specific amino acid positions in peptides are available for T cell recognition.

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Skipper et al. ((1996) *J. Exp. Med.* 183: 527) described the identification and characterization of a naturally-occurring peptide epitope of tyrosinase wherein the peptide sequence differed from that which is predicted from the DNA. This modified peptide was recognized by tyrosinase-specific human cytotoxic T-lymphocytes  
5 ("CTL") more effectively than the direct translation product, and was the only one of the two peptides to be presented by HLA-A2.1 molecules on the cell surface. The modification was a substitution of an asparagine with an aspartic acid. The authors proposed that the asparagine was N-glycosylated in the endoplasmic reticulum during protein synthesis and subsequently deamidated post-translationally.

10

With respect to the CEA epitope CAP1, the primary and secondary anchors positions for HLA binding at positions 2, 9, and 1 (of the epitope) are already occupied by preferred amino acids. As such, Schlam and colleagues attempted to increase CAP1 immunogenicity via the generation of epitope analogs containing  
15 single amino acid substitutions to residues predicted to interact with the T cell receptor ("TCR") of CAP1-specific CTL. One such analog epitope (YLSGADLNL; designated CAP-1-6D) was identified which demonstrated an increased immunogenicity to that of the natural epitope, but not a concomitant increase in MHC binding *per se* (i.e. it behaved as an agonist; Zaremba, S. et al (1997) *Supra*).

20

The present invention discloses novel modified CEA agonist polypeptides/proteins containing a modified epitope therein, nucleic acids coding therefor, vectors comprising said nucleic acids, mixtures and/or compositions of the aforementioned agents, and their advantageous use in generating CEA-specific  
25 immune responses and/or in the treatment of cancers.

#### **SUMMARY OF THE INVENTION**

The present invention encompasses novel modified CEA agonist polypeptides/proteins comprising a modified epitope containing the sequence  
30 YLSGADLNL, nucleic acids coding therefore, vectors (such as recombinant virus and/or bacteria) and/or cells (such as antigen-presenting cells) comprising said nucleic acids, and mixtures and/or compositions of the aforementioned. All of these

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aforementioned agents, mixtures and compositions are characterized by their ability to induce or elicit an immune response against a CEA protein or fragment thereof, a CEA agonist polypeptide containing said modified epitope, a normal or modified CEA epitope, or cells binding or expressing the aforementioned CEA  
5 protein/fragment, CEA agonist polypeptide, or normal/modified CEA epitope.

Accordingly, in one embodiment of the invention a CEA agonist polypeptide/protein is provided comprising a modified epitope of CEA, wherein said modified epitope contains the sequence YLSGADLNL.

10

In a further embodiment of the invention, the CEA agonist polypeptide/protein has the amino acid sequence of SEQ ID NO: 1 (Figure 1).

As previously noted, embodiments of the invention encompass nucleic acids  
15 coding for the aforementioned CEA agonist polypeptides/proteins. Accordingly, embodiments of the invention consist/comprise the nucleic acid sequence of SEQ ID NO: 2 (Figure 1). In further embodiments of the invention, the nucleic acid is a DNA selected from the group consisting of viral nucleic acid, plasmid, bacterial DNA, naked/free DNA, and RNA. In yet further embodiments, the viral nucleic acid  
20 is selected from the group consisting of adenovirus, alphavirus and poxvirus. In still yet further embodiments, the poxvirus is selected from the group consisting of avipox, suipox and orthopox. In still yet further embodiments, the poxviral nucleic acid is selected from the group consisting of TROVAC, NYVAC, ALVAC, MVA, Adeno-Associated Virus (AAV), Wyeth; and PoxvacTC.

25

Additional embodiments of the invention are contemplated encompassing nucleic acids comprising a sequence encoding for the CEA agonist polypeptide/protein in addition to a second sequence encoding at least one member selected from the group comprising cytokines, lymphokines, and co-stimulatory  
30 molecules.

Embodiments of the invention further contemplate vectors comprising the

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nucleic acid(s) of the invention. In particular embodiments, these vectors may be either recombinant viruses or bacteria. In further embodiments, the recombinant viruses are selected from the group consisting of adenovirus, alphavirus and poxvirus. In yet further embodiments, the poxvirus is selected for the group  
5 consisting of avipox, orthopox and suipox; particular embodiments encompass ALVAC, NYVAC, TROVAC, MVA, Wyeth and Poxvac-TC.

The invention further provides for cells comprising the aforementioned nucleic acid(s) of the invention, wherein said cells express the CEA agonist  
10 polypeptide/protein of the invention. In further embodiments, the cells expressing the CEA agonist polypeptide/protein also express a MHC HLA class 1 molecule. In yet further embodiments, the cells expressing the polypeptide are antigen-presenting cells.

15 Embodiments of the invention further encompass mixtures and/or compositions of the aforementioned CEA agonist polypeptides/proteins, nucleic acids, vectors, and cells. These mixtures and/or compositions, may optionally include adjuvants.

20 The invention further provides a method of inducing an immune response in an animal directed against:

- (i) a CEA protein or fragment thereof; and/or
- 25 (ii) a CEA agonist polypeptide/protein of the invention; and/or
- (iii) a CEA epitope; and/or
- (iv) a modified CEA epitope; and/or
- 30 (v) cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide/protein of the invention, CEA epitope, modified CEA

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epitope; and/or;

- 5 (vi) cells binding a CEA protein or fragment thereof, CEA agonist polypeptide/protein of the invention, CEA epitope, modified CEA epitope,

comprising administering to said animal a CEA agonist polypeptide/protein, nucleic acid, vector, cell, or mixture and/or composition of the invention in an amount sufficient to induce an immune response.

10 The invention further contemplates a method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of a CEA agonist polypeptide/protein, nucleic acid, vector, cell, or mixture and/or composition of the invention.

15 The invention in yet a further aspect provides for a treatment for cancer comprising any one of the aforementioned methods for inducing immune responses and/or inhibiting carcinoma cells expressing a CEA epitope.

20 Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating particular embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed, description.

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#### **BRIEF DESCRIPTION OF THE FIGURES**

The present invention will be further understood from the following description with reference to the figures, in which:

30 Figure 1 depicts the nucleic acid and amino acid sequence of an embodiment of the invention encompassing modified CEA.

Figure 2 depicts 3 schematic representations encompassing the genomic structure of particular recombinant fowlpox, vaccinia and MVA

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constructs expressing modified CEA.

Figure 3 depicts a schematic representation of the *Xhol* restriction map profile of an ALVAC(2)-CEA (modified)/human B7.1 construct.

5 Figure 4 depicts the nucleic acid sequence of the H6-promoted CEA (modified)/human B7.1 insertion cassette used in the production of the construct of Figure 3.

Figure 5 depicts the results of an immunoprecipitation analysis of HeLa cells infected with various ALVAC recombinant constructs.

10 Figure 6 depicts the results of a western blot analysis of HeLa cells infected with various ALVAC recombinant constructs.

#### **DETAILED DESCRIPTION OF THE INVENTION**

The invention discloses CEA agonist polypeptides/proteins comprising a modified CEA epitope wherein said modified CEA epitope comprises the sequence  
15 YLSGADLNL, nucleic acids coding therefor, vectors and/or cells comprising said nucleic acids (collectively designated as "agents" of the invention), and mixtures/compositions of the aforementioned. All of the aforementioned agents and mixtures/compositions of the invention have the ability to induce or elicit an immune response against a CEA protein fragment thereof, a CEA agonist  
20 polypeptide/protein of the invention, a CEA epitope and/or modified CEA epitope, and/or cells binding or expressing the aforementioned. An "immune response" is defined as any response of the immune system, for example, of either a cell-mediated (i.e. cytotoxic T-lymphocyte mediated) or humoral (i.e. antibody mediated) nature. As is known to those skilled in the art, various  
25 assays/methodologies exist for the assessment and/or monitoring of immunological responses.

Within the context of cell-mediated immune responses, tumor associated antigen proteins (such as CEA) are processed by intracellular proteases into smaller epitope peptides which are subsequently transported to the cell surface tightly bound in a cleft on an MHC HLA class I molecule. T cells recognize these small epitope peptides only when presented in association with MHC HLA Class I molecules on

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the surface of appropriate cells. Analogously, in the context of humoral immune responses proteins can be processed into smaller epitope peptides which are subsequently presented on cell surfaces (i.e. antigen presenting cells) in association with MHC HLA class II molecules. Said complexes are recognized by appropriate 5 cells of the humoral immune system.

As is well known to those skilled in the art, short peptides (i.e. epitopes) composed of amino acid sequences of about 8 to 12 amino acids derived from antigens are capable of binding directly within the cleft of an HLA class I molecule 10 without intracellular processing. As previously noted, a number of such epitope peptides derived from CEA have been identified. Moreover, some of these CEA-specific antigen epitopes have demonstrated the capacity to induce/elicit immune responses wherein appropriate CEA expressing target cells are lysed. The CEA agonist polypeptides/proteins of the present invention (comprising a modified CEA 15 epitope) elicit an improved immune' response (hence, deemed to be CEA "agonist(s)") by comparison to that observed when normal/unmodified CEA (comprising normal epitopes) is employed as an immunogen.

As encompassed by this invention, the CEA agonist polypeptides/proteins 20 comprise a modified epitope containing the amino acid sequence YLSGADLNL (designated "CAP16D"). The counterpart sequence of the naturally occurring epitope in unmodified CEA is YLSGANLNL (designated "CAP1"; Zaremba, S. et al. (1997) *Cancer Res.* 57:4570). In a particular embodiment of the invention, the CEA agonist polypeptide/protein has the amino acid sequence of SEQ ID NO: 1 25 (Figure 1). It is further recognized that CEA agonist polypeptide(s)/protein(s) embodiments of the invention encompass both precursor and mature forms of polypeptide/protein (for example, see Oikawa, S. et al. (1987) *Biochem. Biophys. Res. Commun.* 142:51 1-518).

30 The CEA agonist polypeptides/proteins of the invention may be prepared using a variety of methods known to one skilled in the art. Accordingly, recombinant DNA methods known to those skilled in the art can be utilized to provide these

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polypeptides. Nucleic acid sequences which encode for the CEA agonist polypeptides/proteins of the invention may be incorporated in a known manner into appropriate expression vectors (i.e. recombinant expression vectors). Possible expression vectors include (but are not limited to) cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses, lentiviruses, herpes viruses, poxviruses), so long as the vector is compatible with the host cell used. The expression "vector is compatible with the host cell" is defined as contemplating that the expression vector(s) contain a nucleic acid molecule of the invention (hereinafter described) and attendant regulatory sequence(s) selected on the basis of the host cell(s) to be used for expression, said regulatory sequence(s) being operatively linked to the nucleic acid molecule. "Operatively linked" is intended to mean that the nucleic acid is linked to regulatory sequence(s) in a manner which allows expression of the nucleic acid. Suitable regulatory sequences may be derived from a variety of sources, including bacteria), fungal, or viral genes. (Forexample, see the regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Selection of appropriate regulatory sequence(s) is dependent on the host cell(s) chosen, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include the following: a transcriptional promoter and enhancer, RNA polymerase binding sequence, or a ribosomal binding sequence (including a translation initiation signal). Depending on the host cell chosen and the expression vector employed, other additional sequences (such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription) may be incorporated into the expression vector.

The aforementioned expression vectors of the invention may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin (which confer resistance to certain drugs),  $\beta$ -galactosidase, chloramphenicol acetyltransferase, or firefly luciferase.

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Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as  $\beta$ -galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. Transformant cells can be  
5 selected with appropriate selection molecules if the selectable marker gene encodes a protein conferring antibiotic resistance (i.e. G418 in context of neomycin resistance). As is known to one skilled in the art, cells that have incorporated the selectable marker gene will survive, while cells which do not have any such incorporated detectable marker will die. This makes it possible to visualize and  
10 assay for expression from recombinant expression vectors of the invention. It will also be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

The recombinant expression vectors may also contain genes which encode a  
15 fusion moiety which provides increased expression of the polypeptides of the invention; increased solubility of the polypeptides of the invention; and/or aids in the purification of a target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant polypeptide to allow separation of the recombinant polypeptide  
20 peptide(s) from the fusion moiety subsequent to purification of the fusion protein.

The CEA agonist polypeptides/proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield (1964) *J. Am. Chem. Assoc.* 65:2149) or synthesis in homogenous solution (Methods of Organic Chemistry, E. Wansch (Ed.) Vol. 15, pts. I and II, Thieme, Stuttgart (1987)).

Additional embodiments of the invention encompass nucleic acids coding for the CEA agonist polypeptides/proteins hereinbefore described. As defined herein,  
30 "nucleic acid(s)" encompass (but is not limited to) viral nucleic acid(s), plasmid(s), bacterial DNA, naked/free DNA and RNA. The nucleic acids encompass both single and double stranded forms. As such, these nucleic acids comprise the relevant base

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sequences coding for the aforementioned polypeptides. For purposes of definitiveness, the “relevant base sequence’s coding for the aforementioned polypeptides” further encompass complementary nucleic acid sequences.

5        In one embodiment of the invention, the nucleic acid has the sequence denoted by SEQ ID NO:2 (Figure 1). In further embodiments of the invention, the nucleic acid comprises this sequence (i.e. SEQ ID NO:2 (Figure 1)). It is further recognized that additional embodiments of the invention may consist of and/or comprise nucleic acid sequences coding for precursor or mature CEA agonist  
10      polypeptide(s)/protein(s) (for example, see Oikawa, S. et al. (1987) *Supra*).

Bacterial DNA useful in embodiments of the invention are known to those of ordinary skill in the art. These bacteria include, for example, *Shigella*, *Salmonella*, *Vibrio cholerae*, *Lactobacillus*, *Bacille Calmette Guérin (BCG)*, and *Streptococcus*.  
15      In bacterial DNA embodiments of the invention, nucleic acid of the invention may be inserted into the bacterial genome, can remain in a free state, or be parried on a plasmid.

20      Viral nucleic acid embodiments of the invention may be derived from a poxvirus or other virus such as adenovirus or alphavirus. Preferably the viral nucleic acid is incapable of integration in recipient animal cells. The elements for expression from said nucleic acid may include a promoter suitable for expression in recipient animal cells.

25      Embodiments of the invention encompass poxviral nucleic acid selected from the group consisting of avipox, orthopox, and suipox nucleic acid. Particular embodiments encompass poxviral nucleic acid selected from vaccinia, fowlpox, canary pox and swinepox; specific examples include TROVAC, NYVAC, ALVAC, MVA, Wyeth and Poxvac-TC (described in more detail below).  
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It is further contemplated that nucleic acids of this invention may further comprise nucleic acid sequences, encoding at least one member chosen from the

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group consisting cytokines, lymphokines, and co-stimulatory molecules. Examples include (but are not limited to) interleukin 2, interleukin 12, interleukin 6, interferon gamma, tumor necrosis factor Alpha, GM-CSF, B7.1, B7.2, ICAM-1, LFA-3, and Cd72.

5

Standard techniques of molecular biology for preparing and purifying nucleic acids well known to those skilled in the art can be used in the preparation of aspects of the invention (for example, as taught in *Current Protocols in Molecular Biology*, F.M. Ausubel et al. (Eds.), John Wiley and Sons, Inc, N.Y., U.S.A. (1998), Chpts. 1, 10 2 and 4; *Molecular Cloning: A Laboratory Manual (2nd Ed.)*, J. Sambrook, E.F. Fritsch and T. Maniatis (Eds.), Cold Spring Harbor Laboratory Press, N.Y., U.S.A. (1989), Chpts. 1, 2, 3 and 7).

Aspects of this invention further encompass vector comprising, the 15 aforementioned nucleic acids. In certain embodiments, said vectors may be recombinant viruses or bacteria.

Adenovirus vectors and methods, for their construction have been described (e.g. U.S. Patent Nos. 5994132, 5932210, 6057158 and Published PCT Applications 20 WO 9817783, WO 9744475, WO 9961034, WO 9950292, WO 9927101, WO 9720575, WO 9640955, WO 9639534, all of which are herein incorporated by reference). Alphavirus vectors have also been described in the art and can be used in embodiments of this invention (e.g. U.S. Patent Nos. 5792462, 5739026, 5843723, 5789245, and Published PCT Applications WO 9210578, WO 9527044, WO 25 9531565, WO 9815636 all of which are herein incorporated by reference), as have lentivirus vectors (e.g. U.S. Patent Nos. 6013516, 5994136 and Published PCT Applications WO 9617816, WO 9712622, WO 9817815, WO 9839463, WO 9846083, WO 9915641, WO 9919501, WO 9930742, WO 9931251, WO 9851810, WO 0000600 all of which are herein incorporated by reference). Poxvirus vectors 30 that can be used include, for example, avipox, orthopox or suipox poxvirus (as described in U.S. Patent Nos. 5364773, 4603112, 5762938, 5378457, 5494807, 5505941, 5756103, 5833975 and 5990091 all of which are herein incorporated by

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reference). Poxvirus vectors comprising a nucleic acid coding for a CEA agonist polypeptide/protein of the invention can be obtained by homologous recombination as is known to one skilled in the art, as such, the nucleic acid coding for the CEA agonist polypeptide/protein is inserted into the viral genome under appropriate 5 conditions for expression in mammalian cells (as described below).

In one embodiment of the invention the poxvirus vector is ALVAC (1) or ALVAC (2) (both of which have been derived from canarypox virus). ALVAC (1) (or ALVAC (2)) does not productively replicate in non-avian hosts, a characteristic 10 thought to improve its safety profile. ALVAC (1) is an attenuated canarypox virus-based vector that was a plaque-cloned derivative of the licensed canarypox vaccine, Kanapox (Tartaglia et al. (1992) *Virology* 188:217; U.S. Patent Nos. 5505941, 5756103 and 5833975 all of which are incorporated herein by reference). ALVAC (1) has some general properties which are the same as some general properties of 15 Kanapox. ALVAC-based recombinant viruses expressing extrinsic immunogens have also been demonstrated efficacious as vaccine vectors (Tartaglia et al., In AIDS Research Reviews (vol. 3) Koff W., Wong-Staol F. and Kenedy R.C. (eds.), Marcel Dekker NY, pp. 361-378 (1993a); Tartaglia, J. et al. (1993b) *J. Virology* 67:2370). For instance, mice immunized with an ALVAC (1) recombinant expressing the rabies 20 virus glycoprotein were protected from lethal challenge with rabies virus (Tartaglia, J. et al., (1992) *supra*) demonstrating the potential for ALVAC (1) as a vaccine vector. ALVAC-based recombinants have also proven efficacious in dogs challenged with canine distemper virus (Taylor, J. et al. (1992) *Virology* 187:321) and rabies virus (Perkus, M.E. et al., In Combined Vaccines and Simultaneous 25 Administration: Current Issues and Perspective, Annals of the New York Academy of Sciences (1994)), in cats challenged with feline leukemia virus (Tartaglia, J. et al., (1993b) *supra*), and in horses challenged with equine influenza virus (Taylor, J. et al., In Proceedings of the Third International Symposium on Avian Influenza, Univ. of Wisconsin-Madison, Madison, Wisconsin, pp. 331-335 (1993)).

30

ALVAC (2) is a second-generation ALVAC vector in which vaccinia transcription elements E3L and K3L have been inserted within the C6 locus (U.S.

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Patent No. 5990091, incorporated herein by reference). The E3L encodes a protein capable of specifically binding to dsRNA. The K3L ORF has significant homology to E1 F-2. Within ALVAC (2) the E3L gene is under the transcriptional control of its natural promoter, whereas K3L has been placed under the control of the early/late 5 vaccine H6 promoter. The E3L and K3L genes act to inhibit PKR activity in cells infected with ALVAC (2), allowing enhancement of the level and persistence of foreign gene expression.

Additional viral vectors encompass natural host-restricted poxviruses.  
10 Fowlpox virus (FPV) is the prototypic virus of the Avipox genus of the Poxvirus family. Replication of avipox viruses is limited to avian species (Matthews, R.E.F. (1982) *Intervirology* 17:42) and there are no reports in the literature of avipox virus causing a productive infection in any non-avian species including man. This host restriction provides an inherent safety barrier to transmission of the virus to other  
15 species and makes use of avipox virus based vectors in veterinary and human applications an attractive proposition.

FPV has been used advantageously as a vector expressing immunogens from poultry pathogens. The hemagglutinin protein of a virulent avian influenza virus was  
20 expressed in an FPV recombinant. After inoculation of the recombinant into chickens and turkeys, an immune response was induced which was protective against either a homologous or a heterologous virulent influenza virus challenge (Taylor, J. et al. (1968) *Vaccine* 6: 504). FPV recombinants expressing the surface glycoproteins of Newcastle Disease Virus have also been developed (Taylor, J. et al.  
25 (1990) *J. Virol.* 64:1441; Edbauer, C. et al. (1990) *Virology* 179:901); U.S. Patent No. 5766599 incorporated herein by reference).

A highly attenuated strain of vaccinia, designated MVA, has also been used as a vector for poxvirus-based vaccines. Use of MVA is described in U.S. Patent No.  
30 5,185,146.

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Other attenuated poxvirus vectors have been prepared via genetic modification to wild type strains of vaccinia. The NYVAC vector, for example, is derived by deletion of specific virulence and host-range genes from the Copenhagen strain of vaccinia (Tartaglia, J. et al. (1992), *supra*; U.S. Patent Nos. 5364773 and 5 5494807 incorporated herein by reference) and has proven useful as a recombinant vector in eliciting a protective immune response against expressed foreign antigens.

Recombinant viruses can be constructed by processes known to those skilled in the art (for example, as previously described for vaccinia and avipox viruses; U.S. 10 Patent Nos. 4769330; 4722648; 4603112; 5110587; and 5174993-all of which are incorporated herein by reference).

In further embodiments of the invention, live and/or attenuated bacteria may also be used as vectors. For example, non-toxicogenic *Vibrio cholerae* mutant 15 strains may be useful as bacterial vectors in embodiments of this invention; as described in US Patent, No. 4,882,278 (disclosing a strain in which a substantial amount of the coding sequence of each of the two ctxA alleles has been deleted so that no functional cholera toxin is produced), WO 92111354 (strain in which the irgA locus is inactivated by mutation; this mutation can be combined in a single 20 strain with ctxA mutations), and WO 94/1533 (deletion mutant lacking functional ctxA and attRS1 DNA sequences). These strains can be genetically engineered to express heterologous antigens, as described in WO 94/19482. (All of the aforementioned issued patent/patent applications are incorporated herein by reference). Attenuated *Salmonella typhimurium* strains, genetically engineered for 25 recombinant expression of heterologous antigens and their use as oral immunogens are described, for example, in WO 92/11361.

As noted, those skilled in the art will readily recognize that other bacterial strains useful as bacterial vectors in embodiments of this invention include (but are 30 not limited to) *Shigella flexneri*, *Streptococcus gordonii*, and *Bacille Calmette Guerin* (as described in WO 88/6626, WO 90/0594, WO 91/13157, WO 92/1796, and WO 92/21376; all of which are incorporated herein by reference). In bacterial

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vector embodiments of this invention, a nucleic acid coding for a CEA agonist polypeptide/protein may be inserted into the bacterial genome, can remain in a free state, or be carried on a plasmid.

5 It is further contemplated that the invention encompasses vectors which comprise nucleic acids coding for at least one member from the group consisting of cytokines, lymphokines and immunostimulatory molecules. Said nucleic acid sequences can be contiguous with sequences coding for CEA agonist polypeptide/proteins, or encoded on distinct nucleic acids.

10

Cells comprising the aforementioned nucleic acids coding for CEA agonist polypeptides/proteins encompass further embodiments of the invention. These cells encompass any potential cell into which the aforementioned nucleic acid might be introduced and/or transfected (for example, bacteria, COS cells, Vero cells, chick 15 embryo fibroblasts, tumor cells, and antigen presenting cells). The choice of process for the introduction and/or transfection into cells is dependant upon the intrinsic nature of the nucleic acid (i.e. free DNA, plasmid, incorporated into a recombinant virus), as will be known to one skilled in the art (for example, as taught in *Current Protocols in Molecular Biology*, F.M. Ausubel et al. (Eds.), John Wiley and Sons, 20 Inc., N.Y., U.S.A. (1998), Chpt. 9; *Molecular Cloning: A Laboratory Manual* (2nd Ed.), J. Sambrook, E.F. Fritsch and T. Maniatis (Eds.), Cold Spring Harbor Laboratory Press, N.Y., U.S.A. (1989), Chpt's. 1,2, 3 and 16).

It is well documented that the class I and class II proteins of the major 25 histocompatibility complex (MHC) perform a central immunological function in focusing T-lymphocytes of the immune system (i.e. CD8+ and CD4+ T lymphocytes). MHC class I proteins are expressed in nearly all nucleated cell types throughout the human body; MHC class II molecules are expressed mainly on antigen-presenting cells (APCs; namely, mononuclear phagocytes, Langerhans- 30 dendritic cells, and B lymphocytes). These distinct classes of cell surface molecules (i.e. class I and class II) present peptides/epitopes (derived from intracellular processing of protein antigens) to T lymphocytes (CD8+ and CD4+ T lymphocytes

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respectively) thus initiating both cellular and humoral immune responses. Generally, epitopes/peptides derived from alloantigens, tumor antigens or viruses will be presented in association, with MHC class I molecules; extracellular antigens/proteins will be presented in association with MHC class II molecules.

5 However, in some contexts endogenous antigens can also be presented in association with MHC class II molecules. [These general immunological principles are well described in the art-as, for example, in *Encyclopedia of Immunology* (2nd Ed.), Peter J. Delves (Ed.-in-Chief), Academic Press, San Diego, U.S.A., pp. 174-8, 191-8, 1108-13, 1690-709(1998).]

10

As such, embodiments of the invention contemplate cells into which has been introduced/transfected a nucleic acid coding for a CEA agonist polypeptide/protein wherein said cells express said polypeptide/protein.

15

As further conceived herein, embodiments of the invention also encompass cells into which has been introduced/transfected a nucleic acid coding for CEA agonist polypeptide/protein wherein said cells also express a MHC HLA molecule (i.e. class I and/or class II). In further embodiments, these cells are antigen-presenting cells, possibly selected from the group consisting of mononuclear 20 phagocytes, Langerhans dendritic cells ("dendritic cell(s)"), and B lymphocytes.

25

Aspects of this invention contemplate mixtures of the CEA agonist polypeptide(s)/protein(s), nucleic acids coding therefor, vectors comprising said nucleic acids, or cells comprising said nucleic acids, and at least one members selected from the group consisting of cytokines, lymphokines, immunostimulatory 30 molecules, and nucleic acids coding therefor. Additional embodiments of this invention further encompass pharmaceutical compositions comprising the CEA agonist polypeptide/protein, nucleic acids coding therefor, vectors cells or mixtures for administration to subjects in a biologically compatible form suitable for administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. Administration of a therapeutically

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active amount of the pharmaceutical compositions of the present invention, or an "effective amount", is defined as an amount effective at dosages and for periods of time, necessary to achieve the desired result of eliciting an immune response in a human. A therapeutically effective amount of a substance may vary according to  
5 factors such as the disease state/health, age, sex, and weight of the recipient, and the inherent ability of the particular polypeptide, nucleic acid coding therefor, or recombinant virus to elicit a desired immune response. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or on at periodic intervals, and/or the dose may be  
10 proportionally reduced as indicated by the exigencies of the therapeutic situation.

The compositions described herein can be prepared by *per se* known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance  
15 (i.e. CEA agonist polypeptide/protein, nucleic acid coding therefor) is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in "Handbook of Pharmaceutical Additives" (compiled by Michael and Irene Ash, Gower Publishing Limited, Aldershot, England (1995)). On this basis, the compositions include, albeit not exclusively, solutions of the substances in  
20 association with one or more pharmaceutically acceptable vehicles or diluents, and may be contained in buffered solutions with a suitable pH and/or be iso-osmotic with physiological fluids. In this regard, reference can be made to U.S. Patent No. 5,843,456. These compositions may further comprise an adjuvant (as described below).

25

Methods of inducing or eliciting an immune response in an animal directed against:

- a CEA protein or fragment thereof; and/or
- a CEA agonist polypeptide/protein of the invention; and/or
- 30 a CEA epitope; and/or
- a modified CEA epitope; and/or
- cells expressing a CEA protein or fragment thereof, CEA agonist

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polypeptide/protein of the invention, CEA epitope, modified CEA epitope; and/or cells binding a CEA protein or fragment thereof, CEA agonist polypeptide/protein of the invention, CEA epitope, modified CEA epitope, comprising the step of; administering to said animal a CEA agonist polypeptide/protein or fragment thereof,

5 a nucleic acid coding therefor, a vector or cell comprising said nucleic acid; mixtures thereof or pharmaceutical compositions of the aforementioned (hereinafter collectively referred to as "immunizing agent's)", "agent's)", or "immunogen(s)" are also within the scope of this invention. As previously noted, an "immune response" is defined as any response of the immune system, for example, of either a

10 cell-mediated (i.e. cytotoxic T-lymphocyte mediated) or humoral (i.e. antibody mediated) nature. These immuhe responses can be assessed by a number of *in vivo* or *in vitro* assays well known to one skilled in the art (for example, (but not limited to) antigen specific cytotoxicity assays, production of cytotoxins, regression of tumors expressing CEA/CEA-epitopes, inhibition of cancer cells expressing

15 CEA/CEA epitopes).

Further embodiments of the invention encompass methods inhibiting a CEA epitope expressing carcinoma cell, in a patient comprising administering to said patient an effective amount of an immunogen of the invention. Patients with solid tumors expressing CEA (or epitopes thereof) include (but are not limited to) those suffering from colon cancer, lung cancer, pancreas cancer, endometrial cancer, breast cancer, thyroid cancer, melanoma, oral cancer, laryngeal cancer, seminoma, hepatocellular cancer, bile duct cancer, squamous cell carcinoma, and prostate cancer. As such, methods of treating patients with cancer *per se* encompassing the aforementioned methods of inducing an immune response and/or inhibiting a CEA epitope expressing carcinoma cell are contemplated aspects/embodiments of the invention.

As known to one of ordinary skill in the art, an animal may be immunized with an immunogen of the invention by any conventional route. This may include, for example, immunization via a mucosal surface (e.g., ocular, intranasal, oral, gastric, pulmonary, intestinal, rectal, vaginal, or urinary tract) or via a parenteral

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route (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal). Preferred routes depend upon the choice of the immunogen (i.e. polypeptide vs. nucleic acid, recombinant/virus, composition formulation, etc.). The administration can be achieved in a single dose or repeated at intervals. The  
5 appropriate dosage is dependant on various parameters understood by the skilled artisans, such as the immunogen itself (i.e. polypeptide vs. nucleic acid (and more specifically type thereof)), the route of administration and the condition of the animal to be vaccinated (weight, age and the like). As such, embodiments of this invention encompass methods of eliciting immune responses in animals comprising  
10 administering an effective amount of a CEA agonist polypeptide/proteins of the invention, a nucleic acid coding therefore, vector or cells or recombinant virus comprising said nucleic acid, mixtures thereof, or pharmaceutical compositions of the aforementioned.

15 As previously noted, nucleic acids (in particular plasmids and/or free/naked DNA and/or RNA coding for the CEA agonist polypeptide/protein of the invention) can be administered to an animal for purposes of inducing/eliciting an immune response (for example, U.S. Patent No. 5589466; McDonnell and Askari, NEJM 334:42-45 (1996), Kowalczyk and Ertl, *Cell Mot Life Sct* 55:751-770 (1999)).

20 Typically, this nucleic acid is a form that is unable to replicate in the target animal's cell and unable to 'integrate in said animal's genome. The DNA/RNA molecule encoding the CEA agonist polypeptide/protein is also typically placed under the control of a promoter suitable for expression in the animal's cell. The promoter can function ubiquitously or tissue specifically. Examples of non-tissue specific  
25 promoters include the early Cytomegalovirus (CMV) promoter (described in U.S. Patent No. 4,168,062) and the Rous Sarcoma Virus promoter. The desmin promoter is tissue-specific and drives expression in muscle cells. More generally, useful vectors have been described (i.e., WO 94/21 797).

30 For administration of nucleic acids coding for a CEA agonist polypeptide/protein of the invention, said nucleic acid can encode a precursor or mature form of the polypeptide/protein. When it encodes a precursor form, the

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precursor form can be homologous (for example, see Oikawa, S. et al. (1987) *Supra* or heterologous. In the latter case, a eucaryotic leader sequence can be used, such as the leader sequence of the tissue-type plasminogen factor (tPA).

5 For use as an immunogen, a nucleic acid of the invention can be formulated according to various methods known to a skilled artisan. First, a nucleic acid can be used in a naked/free form, free of any delivery vehicles (such as anionic liposomes, cationic lipids, microparticles, (e.g., gold microparticles), precipitating agents (e.g., calcium phosphate) or any other transfection-facilitating agent. In this case the  
10 nucleic acid can be simply diluted in a physiologically acceptable solution (such as sterile saline or sterile buffered saline) with or without a carrier. When present, the carrier preferably is isotonic, hypotonic, or weakly hypertonic, and has a relatively low ionic strength (such as provided by a sucrose solution (e.g., a solution containing 20% sucrose)).

15 Alternatively, a nucleic acid can be associated with agents that assist in cellular uptake. It can be, i.e., (i) complemented with a chemical agent that modifies the cellular permeability (such as bupivacaine; see, for example, WO 94/16737), (ii) encapsulated into liposomes, or (iii) associated with cationic lipids or silica, gold, or  
20 tungsten microparticles.

Cationic lipids are well known in the art and are commonly used for gene delivery. Such lipids include Lipofectin (also known as DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOTAP (1,2-bis(oleyloxy)-3-(trimethylammonio)propane), DDAB (dimethyldioctadecylammonium bromide), DOGS (dioctadecylamidoglycyl spermine) and cholesterol derivatives such as DC-Chol (3 beta-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol). A description of these cationic lipids can be found in EP 187,702, WO 90/11092, U.S. Patent No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Patent No. 5,527,928. Cationic lipids for gene delivery are preferably used  
25 in association with a neutral lipid such as DOPE (dioleyl phosphatidylethanolamine), as for example, described in WO 90/11092.

Other transfection-facilitating compounds can be added to a formulation containing cationic liposomes. A number of them are described in, for example, WO 93/18759, WO 93/19768, WO 94/25608, and WO 95/2397. They include, i.e.,  
5 Spermine derivatives useful for facilitating the transport of DNA through the nuclear membrane (see, for example, WO 93/18759) and membrane-permeabilizing compounds such as GALA, Gramicidine 5, and cationic bile salts (see, for example, WO 93/19768).

10 Gold or tungsten microparticles can also be used for gene delivery (as described in WO 91/359 and WO 93/17706). In this case, the microparticle-coated polynucleotides can be injected via intradermal or intraepidermal routes using a needleless injection device ("gene gun"), such as those described, for example, in U.S. Patent No. 4,945,650, U.S. Patent No. 5,015,580, and WO 94/24263.

15 Anionic and neutral liposomes are also well-known in the art (see, for example, Liposomes: A Practical Approach, RPC New Ed, IRL Press (1990), for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including nucleic acids.

20 Particular embodiments of the aforementioned methods (i.e. to induce/elicit immune responses and/or to inhibit a CEA epitope expressing carcinoma cell in a patient) encompass prime-boost protocols for the administration of immunogens of the invention. More specifically, these protocols encompass (but are not limited to) a  
25 "priming" step with a particular/distinct form of immunogen (i.e. nucleic acid (for example, plasmid, bacterial/viral/free or naked) coding for an immunogen, or vector (i.e. recombinant virus, bacteria) comprising said nucleic acid) followed by at least one "boosting" step encompassing the administration of an alternate (i.e. distinct from that used to "prime") form of the immunogen (i.e. protein or fragment thereof  
30 (for example, epitope-peptide), nucleic acid coding for an immunogen (or fragment thereof), or vector comprising said nucleic acid). Examples of "prime-boost" methodologies are known to those skilled in the art (as taught, for example, in PCT

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published applications WO 00/00216, WO 98/58956, WO 98/56919, WO 97/39771). One advantage of said protocols is the potential to circumvent the problem of generating neutralizing immune responses to viral vectors *per se* wherein is inserted incorporated nucleic acids encoding the immunogen or fragments thereof  
5 (see for example, R.M. Conty et al. (2000) *Clin. Cancer Res.* 6:34-41).

As is well known to those of ordinary skill in the art, the ability of an immunogen to induce/elicit an immune response can be improved if, regardless of administration formulation (i.e. recombinant virus, nucleic acid, polypeptide), said  
10 immunogen is coadministered with an adjuvant. Adjuvants are described and discussed in “Vaccine Design-the Subunit and Adjuvant Approach” (edited by Powell and Newman, ‘Plenum Press, New York, U.S.A., pp. 61-79 and 141-228 (1995)). Adjuvants typically enhance the immunogenicity of an Immunogen but are not necessarily immunogenic in and of themselves. Adjuvants may act by retaining  
15 the immunogen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of immunizing agent to cells of the immune system. Adjuvants can also attract cells of the, immune system to an immunogen depot and stimulate such cells to elicit immune responses. As such, embodiments of this invention encompass compositions further comprising adjuvants.  
20

- Desirable characteristics of ideal adjuvants include:
- 1) lack of toxicity;
  - 2) ability to stimulate a long-lasting immune response;
  - 3) simplicity of manufacture and stability in long-term storage;
  - 25 4) ability to elicit both cellular and humoral responses to antigens administered by various routes, if required;
  - 5) synergy with other adjuvants;
  - 6) capability of selectively interacting with populations of antigen presenting cells (APC);
  - 30 7) ability to specifically elicit appropriate TRI or TH2 cell-specifib immune responses; and
  - 8) ability to selectively increase appropriate antibody isotype levels (for

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example, IgA) against antigens/immunogens.

However, many adjuvants are toxic and can cause undesirable side effects, thus making them unsuitable for use in humans and many animals. For example, some  
5 adjuvants may induce granulomas, acute and chronic inflammations (i.e. Freund's complete adjuvant (FCA)), cytolysis (i.e. saponins and pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (i.e. muramyl dipeptide (MDP) and lipopolysaccharide (LPS)). Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as  
10 adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established. Notwithstanding, it does have limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response with other immunogens. The antibodies elicited by alum-adjuvanted antigens are  
15 mainly of the IgG1 isotype in the mouse, which may not be optimal for protection in vaccination contexts.

Adjuvants may be characterized as "intrinsic" or "extrinsic". Intrinsic adjuvants (such as lipopolysaccharides) are integral and normal components of agents which in  
20 themselves are used as vaccines (i.e. killed or attenuated bacteria). Extrinsic adjuvants are typically nonintegral immunomodulators generally linked to antigens in a noncovalent manner, and are formulated to enhance the host immune response.

In embodiments of the invention, adjuvants can be at least one member chosen  
25 from the group consisting of cytokines, lymphokines, and co-stimulatory molecules. Examples include (but are not limited to) interleukin 2, interleukin 12, interleukin 6, interferon gamma, tumor necrosis factor alpha, GM-CSF, 87.1, 87.2, ICAM-1, LFA-3, and CD72. Particular embodiments specifically encompass the use of GM-CSF as  
30 an adjuvant (as taught, for example, in U.S. Patent Nos. 5679356, 5904920, 5637483, 5759535, 5254534, European Patent Application EP 211684, and published PCT document WO 97/28816 all of which are herein incorporated by reference).

A variety of potent extrinsic adjuvants have been described. These include (but are not limited to) saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvant, bacterial products such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

The use of saponins *per se* as adjuvants is also well known (Lacaille-Dubois, M. and Wagner, H (1996) *Phytomedicine* 2:363). For example, Quil A (derived from the bark of the South American tree, Quillaja Saponaria Molina) and fractions thereof has been extensively described (i.e. U.S. Patent No. 5057540; Kensil, C.R. (1996) *Crit Rev Ther Drug Carrier Syst* 12:1; and European Patent EP 362279). The haemolytic saponins Q521 and QSI7 (HPLC purified fractions of Quil A) have been described as potent systemic adjuvants (U.S. Patent No. 5057540: European Patent EP 362279). Also described in these references is the use of QS7 (a non-haemolytic fraction of Quil-A) which acts as a potent adjuvant for systemic vaccines. Use of QS2I is further described in Kensil et al. ((1991) *J. Immunol* 146:431). Combinations of O521 and polysorbate or cyclodextrin are also known (WO 9910008). Particulate adjuvant systems comprising fractions of Quil A (such as QS2I and QS7) are described in WO 9633739 and WO 9611711.

Another preferred adjuvant/immunostimulant is an immunostimulatory oligonucleotide containing unmethylated CpG dinucleotides ("CpG"). CpG is an abbreviation for cytosine-guanosine dinucleotide motifs present in DNA. CpG is known in the art as being an adjuvant when administered by both systemic and mucosal routes (WO 9602555; European Patent EP 468520; Davies et al. (1998). *J. Immunol.* 160:87; McCluskie and Davis (1998) *J. Immunol* 161:4463). In a number of studies, synthetic oligonucleotides derived from BCG gene sequences have also been shown to be capable of inducing immunostimulatory effects (both *in vitro* and *in vivo*; Krieg, (1995) *Nature* 374:546). Detailed analyses of immunostimulatory oligonucleotide sequences has demonstrated that the CG motif must be in a certain sequence context, and that such sequences are common in bacterial DNA but are

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rare in vertebrate DNA. (For example, the immunostimulatory sequence is often: purine, purine, C, G, pyrimidine, pyrimidine, wherein the CG motif is not methylated; however other unmethylated CpG sequences are known to be immunostimulatory and as such may also be used in the present invention.) As will 5 be evident to one of normal skill in the art, said CG motifs/sequences can be incorporated into nucleic acids of the invention *per se*, or reside on distinct nucleic acids.

A variety of other adjuvants are taught in the art, and as such are encompassed 10 by embodiments of this invention. U.S. Patent No. 4,855,283 granted to Lockhoff et al. (incorporated herein by reference) teaches glycolipid analogues and their use as adjuvants. These include N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Furthermore, Lockhoff et al. ((1991) 15 *Chem. mt. Ed. Engl.* 30:1611) have reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids (such as glycophospholipids and glycoglycerolipids) are also capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine.

20

U.S. Patent No. 4,258,029 granted to Moloney (incorporated herein by reference) teaches that octadecyl tyrosine hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus vaccine. Nixon-George et al. ((1990) *J. Immunol.* 14:4798) 25 have also reported that octadecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen enhanced the host immune responses against hepatitis B virus.

Adjuvant compounds may also be chosen from the polymers of acrylic or 30 methacrylic acid and the copolymers of maleic anhydride and alkenyl derivative. Adjuvant compounds are the polymers of acrylic or methacrylic acid which are cross-linked, especially with polyalkenyl ethers of sugars or polyalcohols. These

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compounds are known by the term carbomer (Pharmeuropa Vol. 8, No. 2, June 1996). Preferably, a solution of adjuvant according to the invention, especially of carbomer, is prepared in distilled water, preferably in the presence of sodium chloride, the solution obtained being at acidic pH. This stock solution is diluted by  
5 adding it to the desired quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with NaCl, preferably physiological saline (NaCl 9g/l) all at once in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4), preferably with NaOH. This solution at physiological pH will be used as it is for mixing with the immunizing agent; said mixture being  
10 amenable to storage in the freeze-dried, liquid or frozen form.

Persons skilled in the art can also refer to U.S. Patent No. 2,909,462 (incorporated herein by reference) which describes adjuvants encompassing acrylic polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups (preferably not more than 8), the hydrogen atoms of the at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing from 2 to 4 carbon atoms (e.g. vinyls, allyls and other ethylenically unsaturated groups). The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under  
15 the name Carbopol (BF Goodrich, Ohio, USA) are particularly appropriate. They are cross-linked with allyl sucrose or with allyl pentaerythritol. Among them, there may be mentioned Carbopol (for example, 974P, 934P and 971 P). Among the copolymers of maleic anhydride and alkenyl derivative, the copolymers EMA (Monsanto; which are copolymers of maleic anhydride and ethylene, linear or cross-linked, (for example cross-linked with divinyl ether)) are preferred. Reference may  
20 be made to J. Fields et al. ((1960) *Nature* 186:778) for a further description of these chemicals (incorporated (herein by reference)).

In further aspects of this invention, adjuvants useful for parenteral  
30 administration of immunizing agent include aluminum compounds (such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate; but might also be a salt of calcium, iron or zinc, or may be an insoluble suspension of

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acylated tyrosine, or acylated sugars, cationically or anionically derivatised polysaccharides, or polyphosphazenes). The antigen can be precipitated with, or adsorbed onto, the aluminum compound according to standard protocols well known to those skilled in the art.

5

Other adjuvants encompassed by embodiments of this invention include lipid A (in particular 3-de-0-acylated monophosphoryl lipid A (3D-MPL)). 3D-MPL is a well known adjuvant manufactured by Ribi Immunochem, Montana. Chemically it is often supplied as a mixture of 3-de-0-acylated monophosphoryl lipid A with 4, 5, or 10 6 acylated chains. It can be prepared by the methods taught in GB 21222048. A preferred form of 3D-MPL is in the form of a particulate formulation having a particle size less than 0.2pm in diameter (European Patent EP 689454).

15 Adjuvants for mucosal immunization may include bacterial toxins (e.g., the cholera toxin (CT), the *E. coli* heat-labile toxin (LT), the *Clostrilium difficile* toxin A and the pertussis toxin (PT), or combinations, subunits, toxoids, or mutants thereof). For example, a purified preparation of native cholera toxin subunit B (CTB) can be of use. Fragments, homologs, derivatives, and fusion to any of these toxins are also suitable, provided that they retain adjuvant activity. A mutant having 20 reduced toxicity may be used. Mutants have been described (e.g., in WO 95/17211 (Arg-7-Lys CT mutant), WO 96/6627 (Arg 192-Gly LT mutant), and WO 95134323 (Arg-9-Lys and Glu-129-Gly PT mutant)). Additional LT mutants include, for example Ser-63-Lys, Ala-69-Gly, Glu-1 10-Asp, and Glu-1 12-Asp mutants. Other adjuvants (such as a bacterial monophosphoryl lipid A (MPLA)) of various sources 25 (e.g., *E. coli*, *Salmonella minnesota*, *Salmonella typhimurium*, or *Shigella flexneri*) can also be used in the mucosal administration of immunizing agents.

30 Adjuvants useful for both mucosal and parenteral immunization include polyphosphazene (for example, WO 95/2415), DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol (for example, U.S. Patent No. 5,283,185 and WO 96/14831) and QS-21 (for example, WO 88/9336).

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Adjuvants/immunostimulants as described herein may be formulated together with carriers, such as for example liposomes, oil in water emulsions, and/or metallic salts including aluminum salts (such as aluminum hydroxide). For example, 3D-MPL may be formulated with aluminum hydroxide (as discussed in EP 689454) 5 or oil in water emulsions (as discussed in WO 9517210); QS2I may be advantageously formulated with cholesterol containing liposomes (as discussed in WO 9633739), in oil water emulsions (as discussed in WO 9517210) or alum (as discussed in WO 9815287). When formulated into vaccines, immunostimulatory oligonucleotides (i.e. CpGs) are generally administered in free solution together with 10 free antigen (as discussed in WO 9602555; McCluskie and Davis (1998) *Supra*), covalently conjugated to an antigen (as discussed in WO 9816247), or formulated with a carrier such as aluminum hydroxide or alum (as discussed in Davies et al. *Supra*; Brazolot-Millan et al (1998) *Proc. Natl. Acad. Sci.* 95:15553).

15 Combinations of adjuvants/immunostimulants are also within the scope of this invention. For example, a combination of a monophosphoryl lipid A and a saponin derivative (as described in WO 9400153, WO 9517210, WO 9633739, WO 9856414, WO 9912565, WO 9911214) can be used, or more particularly the combination of QS2I and 3D-MPL (as described in WO 9400153). A combination 20 of an immunostimulatory oligonucleotide and a saponin (such as QS2I), or a combination of monophosphoryl lipid A (preferably 3D-MPL) in combination with an aluminum salt also form a potent adjuvant for use in the present invention.

25 The following non-limiting examples are illustrative of the present invention:

#### EXAMPLES

##### Example 1: Generation of a Modified CEA Gene

T-cell epitopes within the native CEA protein include CAP-1; said epitope 30 comprising the amino acid sequence YLSGANLN (Tsang et al. (1995) *J. Natl. Cancer Inst* 87:982-990). As previously noted, the immunogenicity of this epitope (in the form of a peptide) was increased by changing position six of the CAP-1

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epitope from N (asparagine) to D (aspartic acid) (Zaremba et al. (1997) *Cancer Res.* 57:4570-4577). Using standard techniques of *in vitro* mutagenesis (Ausubel et al. (1997) *Curr. Protocols in Mol. Biol.*), this modification was introduced into the full-length CEA gene via mutation of the codon AAC (which encodes asparagine) to 5 GAC (which encodes aspartic acid). The resulting modified CEA gene now comprises the modified epitope YLSGADLNL (the change from N to D is shown in bold). This modified CEA gene, encoding for a protein with aspartic acid (D) in place of asparagine (N) in the sixth amino acid position of the CAP-1 epitope, is designated CEA (6D) (for example, see SEQ ID NO: 1 (Figure 1)).

10

Example 2: Generation of Recombinant Fowlpox, Vaccinia and MVA Constructs

The generation of recombinant poxviruses is accomplished via homologous recombination between poxvirus genomic DNA and a plasmid vector that carries the heterologous sequences to be inserted. Plasmid vectors for the insertion of foreign 15 sequences into poxviruses are constructed by standard methods of recombinant DNA technology (for example, Ausubel et al. (1997) *Curr. Protocols. Mol. Biol.*). The plasmid vectors contain one or more chimeric genes, each comprising a poxvirus promoter linked to a protein coding sequence, flanked by viral sequences from a non-essential region of the poxvirus genome. The plasmid is transfected into 20 cells infected with the parental poxvirus, and recombination between poxvirus sequences on the plasmid and the corresponding DNA in the viral genome results in the insertion into the viral genome of the chimeric genes on the plasmid. Recombinant viruses are selected and purified using any of a variety of selection or screening systems (Mazzara et al. (1993) *Meth. Enzymol.* 217:557-581; Jenkins et al. 25 (1991) *AIDS Res. Hum. Retroviruses* 7:991-998; Sutter et al. (1994) *Vaccine* 12:1032-1040). Insertion of the foreign genes into the vaccinia genome is typically confirmed by polymerase chain reaction (PCR) analysis. Expression of the foreign genes is demonstrated by Western analysis and/or via immunoprecipitation of expressed products.

### Generation of Recombinant Poxviruses

For the generation of recombinant fowlpox virus rF-CEA(6D), a plasmid vector designated pT5071 was constructed to direct insertion of the foreign sequences into the BamH1 J region of the fowlpox genome. The CEA(6D) gene is under the control of the vaccinia 40K promoter (Gritz et al. (1990) *J. Virol.* 64:5948-5957). In addition, the *E. coli lacZ* gene, under the control of the fowlpox virus C1 promoter (Jenkins et al. (1991) *AIDS Res. Hum. Retroviruses* 7:991-998), is included as a screen for recombinant progeny. These foreign sequences are flanked by DNA sequences from the BamHI J region of the fowlpox genome. A plaque-purified isolate from the POXVAC-TC strain of fowlpox was used as the parental virus for this recombinant vaccine. The generation of recombinant fowlpox virus was accomplished via homologous recombination between fowlpox sequences in the fowlpox genome and the corresponding sequences in pT5071 in fowlpox-infected primary chick embryo dermal cells transfected with pT5071. Recombinant virus was identified using a chromogenic assay performed on viral plaques *in situ*. This assay detects expression of the *lacZ* gene product in the presence of halogenated indolyl-beta-D-galactoside (Bluo-gal) (Chakrabarti et al. (1985) *Mol. Cell Bio* 3403-3409). Viral plaques expressing *IacZ* appear blue against a clear background. Positive plaques, designated vT233, were picked from the cell monolayer and their progeny replated. Seven rounds of plaque isolation and replating in the presence of Bluo-Gal resulted in the purification of the desired recombinant. A schematic representation of the genomic structure of vT233 is depicted in Figure 2(A).

For the generation of recombinant vaccinia rV-CEA(6D), a plasmid vector designated pT2 146 was constructed to direct insertion of the foreign sequences into the thymidine kinase gene (located in the Hind III J region of the vaccinia genome). The CEA(6D) gene is under the transcriptional control of the vaccinia 40K promoter and the *E. coli lacZ* gene is under the control of the fowlpox virus Ci promoter. These foreign sequences are flanked by DNA sequences from the Hind III J region of the vaccinia genome. A plaque-purified isolate from the Wyeth (New York City Board of Health) strain of vaccinia was used as the parental virus for this recombinant vaccine. The generation of recombinant vaccinia virus was

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accomplished via homologous recombination between vaccinia sequences in the Wyeth vaccinia genome and the corresponding sequences in pT2146 in vaccinia-infected cells transfected with pT2146. Recombinant virus was identified using the chromogenic assay for the *lacZ* gene product (previously described). Positive 5 plaques, designated vT237, were picked from the cell monolayer and their progeny replated. Five rounds of plaque isolation and replating in the presence of Bluo-Gal resulted in the purification of the desired recombinant. A schematic representation of the genomic structure of vT237 is depicted in Figure 2(6).

10 For the generation of a recombinant MVA that expressed CEA(6D), a plasmid vector was constructed to direct insertion of the foreign sequences into the MVA genome. The CEA(6D) gene and the *E. coli* *lacZ* gene were each under the control of a poxviral promoter. These foreign sequences were flanked by DNA sequences from the MVA genome into which the foreign sequences were to be 15 inserted (for example, deletion III (Sutter et al (1994) Vaccine 12:1032-1040)). The generation of recombinant MVA was accomplished via homologous recombination between MVA sequences in the MVA genome and the corresponding sequences in the plasmid vector in MVA-infected cells transfected with the plasmid vector. Recombinant plaques were picked from the cell monolayer under selective 20 conditions and their progeny further propagated. Additional rounds of plaque isolation and replating resulted in the purification of the desired recombinant virus. A schematic representation of the genomic structure of the MVA recombinant construct is depicted in Figure 2(C).

25 Example 3: Generation of an ALVAC(2)-modified CEA-B7.1 (Human) Recombinant Construct frCP1586

To generate ALVAC(2)-CEAmod/hB7.1, a coding sequence containing the H6 (Perkins et al. (1989) *J. Virol.* 63:3829-3936) promoted modified CEA and the human 67.1 gene (under the control of synthetic Early/Late promoter) was 30 subcloned into a generic ALVAC donor plasmid specific for the C5 insertion site (see below). The donor plasmid was then used to insert the expression cassette into

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the CS insertion locus in the ALVAC(2) genome by *in vitro* recombination (Piccini et al. (1987) *Meth. Enzymol.* 153:545).

The locus designated CS was used for the insertion of the modified CEA and  
5 human B7.1 coding sequences into the ALVAC(2) vector. By virtue of the C5 locus  
existing within the extensive inverted terminal repetitions (ITRs) of the virus  
genome, insertion into this locus results in the occurrence of two copies of the  
inserted sequence. A schematic of the redombinant construct is depicted in Figure 3.  
[Presently, no function has been ascribed to the CS encoded polypeptide nor does  
10 the deduced amino acid open reading frame encoded in this region share significant  
homology to any entry in the existing protein databases.]

***Particulars of the Generation of the vCPIS86 ALVAC recombinant***

15 (I) Modified CEA:  
sequence (up to the Bg/II site) by PCR amplification from plasmid pT2147  
(comprising a full length sequence of the modified CEA(6D)) using the following  
PCR primers:

20 HM102: 5' -TTG-TCC-GAG-CTC-TCG-CGA-TAT-CCG-TTA-AGT-TTG-  
TAT CGT-AAT-GGA-GTC-TCC-CTC-GGC-CCC-3' (Sequence ID  
No. 3)

HM103: 5'-CCG-GAA-TTC-TCA-CAA-GAT-CTG-ACT-TTA-TGA-C-3'  
25 (Sequence ID NO. 4)

The PCR product was digested with *Sac*I and *Eco*R1 to generate compatible  
ends for cloning into *Sac*I, *Eco*R1 and Shrimp Alkaline Phosphatase digested pBSK+  
vector (DH5 $\alpha$  cells). The resulting plasmid (designated pF102.103) was sequenced  
30 to confirm the fidelity of the insert. The remainder of the CEA(6D) sequence was  
isolated by digestion of plasmid pT2147 with *Bg*/II and *Sa*I. The resulting 1.7Kbp  
fragment contained the 3'end of CEA(6D). This fragment was ligated to *Bg*/II, *Sa*I

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and Shrimp Alkaline Phosphatase digested pF102.103 plasmid and transformed into DH5 $\alpha$  cells. The resulting plasmid was designated p3'H6MCEA.

An ALVAC donor plasmid was constructed by transferring the 3'H6-modified CEA sequence fragment from p3'H6MCEA to ALVAC insertion plasmid NVQH6MC5. NVQH6MC5 was initially made via digestion of CS donor plasmid NVQH6C5LSP-18 within its polylinker region with *BamHI*. It was subsequently treated with alkaline phosphatase and ligated to kinased and annealed oligonucleotides SPCSPL1  
5 (5'-GAT-CGT-CGA-CGA-GCT-CGA-ATT-CG-3') (Sequence ID No. 5) and SPC5PL2 (5'-GAT-CCG-AATTCG-ACC-TCG-TCG-AC-3') (Sequence ID No. 6) - thus generating plasmid NVQH6MC5.  
10

Plasmid p3'H6MCEA was digested with *NruI* and *SaII*; the fragment containing the 3'H6 modified CEA sequence purified and subsequently ligated to *NruI*, *XhoI* and Shrimp Alkaline Phosphatase digested NVQH6MC5 vector. The resulting donor plasmid, containing the modified CEA coding sequences under the control of a full length H6 promoter, was designated pAH6MCEA.  
15

20 (ii) B7.1

The following primers were used to fuse the 42K promoter and PCR out the entire B7.1 gene from plasmid pT2147:

25 H M1 04: 5'-TTG-TCC-GAG-CTC-GAA-TTC-TTT-ATT-G GG-AAG-AAT-ATG-ATA-ATA-TTT-TGG-GAT-TTC-AAA-ATT-GAA-AAT-ATA-TAA-TTACAA-TAT-AAA-ATG-GGC-CAC-ACA-CGG-AGG-CAG-3' (Sequence ID No. 7).  
HM105: 5'-ACG-GCA-GTC-GAC-TTA-TAC-AGG-GCG-TAC-ACT-3' (Sequence ID No. 8).  
30

Primer HM1O4 contained the entire sequence of the 42K promoter. The resulting PCR product (designated F104.105) was digested with *EcoRI* and *SaI*, and

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subsequently ligated into *Eco*RI, *Sa*II and Shrimp Alkaline Phosphatase digested pBSK+ vector(SURE cells), The resulting plasmid was designated pF104.105 and was sequenced to confirm the fidelity of the insert.

5 (iii) Donor Plasmid pA2147

The fragment containing the 42K-67.1 coding sequences was excised from pF104.105 by digestion with *Eco*RI and *Sa*I, and subsequently purified. This fragment was ligated to *Eco*RI, *Sa*I, Shrimp Alkaline Phosphatase digested 10 pAH6MCEA plasmid and transformed into SURE cells. The resulting donor plasmid was designated pA2147. (See Figure 4 for the sequence of the H6-promoted human modified CEA/42K promoted B7.1 cassette of pA2147 for insertion into ALVAC.)

15 (iv) Generation of VCP1586

Recombination between donor plasmid pA2147 and ALVAC(2) rescuing virus generated recombinant virus vCP1586. This recombinant viral construct comprised the vaccinia H6 promoted modified human CEA gene sequence and the 20 42K promoted human 67.1 gene sequence in the C5 locus (for ALVAC insertion and inserted DNA sequence particulars, see Figures 3 and 4). Recombination was performed utilizing procedures described in the art and known to skilled artisans (Piccini et al. (1987), *supra*; other procedures can also be utilized as described above and/or as described, for example, in U.S. Patent Nos. 4769330, 4722848, 4603112, 25 5174993, 5110587, all of which are incorporated herein by reference).

*Verification of insertion*

(I) Restriction Enzyme Analysis

30

Viral genomic DNA was isolated from cells infected with vCP1586 pursuant to methods well known to those skilled in the art (for example, as taught in *Current*

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Protocols in Molecular Biology, F.M. Ausubel et al. (Eds.), John Wiley and Sons, Inc., N.Y., U.S.A. (1998); Molecular Cloning: A Laboratory Manual (2<sup>nd</sup> Ed.), J. Sambrook, E.F. Fritsch and T. Maniatis (Eds.), Cold Spring Harbor Laboratory Press, N.Y., U.S.A. (1989)). The viral genomic DNA was digested with restriction endonucleases (*HindIII*, *Pst I*, *BamH1* or *Xhol*). The resultant DNA fragments were fractionated by electrophoresis through an agarose gel and visualized by ethidium bromide staining. The insertion of the modified CEA and B7.1 expression cassette at the CS locus was confirmed (for example, see Figure 3 for a schematic representation of the *Xhol* restriction map of vCPI 586).

10

(ii) Immunoprecipitation

Immunoprecipitation analyses were performed using radiolabeled lysates derived from uninfected HeLa cells or cells infected with either ALVAC(2) parental virus (vCP1486), ALVAC-CEA/B7 (vCP3O7), ALVAC-hB7.1 (vCP11334), ALVAC-CEAmod/hB7.1 (vCP1585) or ALVAC(2)-CEAmod/hB7.1 (vCP1586) as previously described (Taylor et al. (1990) *J. Virol.* 64:1441-1450). Briefly, HeLa cell cultures were infected at a multiplicity of infection (m.o.i.) of 10 pfu/cell in methionine and cystine-free media supplemented with [<sup>35</sup>S]-methionine/cysteine (30 $\mu$ Ci/ml). Cells were lysed at 18 hours post-infection. Immunoprecipitation was performed using a B7.1 specific monoclonal antibody (HB71; ATCC#80991). immunoprecipitates were fractionated on a 10% SDS-Polyacrylamide gel. The gel was fixed and treated for fluorography with 1 M Na-salicylate for 1/2 hr. The dried gel was exposed to Kodak XAR-2 film to visualize the protein species. Results with anti-hB7.1 demonstrate expression of human B7 in HeLa cells infected with ALVAC(2)-CEAmod/hB7.1 (vCP1586) and the other ALVAC-based recombinants expressing hB7.1, but not in parentally infected cells (Figure 5).

(iii) Western Blot Analysis

30

HeLa cells were infected for 18 hours at a multiplicity of 10 pfu/cell with either ALVAC(2) parental virus (vCP1486), ALVAC-CEA/B7 (vCP3O7), ALVAC-

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CEA (vCP248), ALVAC-CEAmod/hB7.1 (vCP 1585) or ALVAC(2)-CEAmod/hB7.1 (vCPI 586). Cell lysates were separated by SDS-PAGE and transferred to nitrocellulose utilizing standard techniques. The blot was incubated with anti-CEA monoclonal antibody Col-1 (1/1 000 dilution), followed by HRP conjugated rabbit anti-mouse utilizing HRP peroxide substrate with DAB/Metal (Pierce). The results obtained demonstrate the expression of full length CEA in HeLa cells infected with ALVAC(2)-CEAmod/hB7.1 (vCP1586) and other ALVAC-based recombinants expressing CEA (Figure 6).

Having illustrated and described the principles of the invention in particular and/or preferred embodiments, it should be appreciated by those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims. All publications, patents and patent applications referred to herein, are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

**CLAIMS****We claim:**

5

1. A CEA agonist polypeptide comprising a modified epitope of CEA, wherein said modified epitope contains the sequence YLSGADLNL.

2. The CEA agonist polypeptide of claim 1 having the sequence of SEQ ID NO: 10 1 (Figure 1).

3. A nucleic acid comprising a nucleic acid sequence which encodes the CEA agonist polypeptide of any one of claims 1 or 2.

15 4. The nucleic acid of claim 3 comprising the sequence of SEQ ID NO: 2 (Figure 1).

5. The nucleic acid of claim 3 having the sequence of SEQ ID NO: 2 (Figure I).

20 6. The nucleic acid of any one of claims 3 through 5 wherein the nucleic acid is selected from viral nucleic acid, bacterial DNA, plasmid DNA, naked/free DNA, and RNA.

25 7. The nucleic acid of claim 6 wherein the viral nucleic acid is selected from the group consisting of adenoviral, alphaviral, and poxviral nucleic acid.

8. The poxviral nucleic acid of claim 7, wherein said nucleic acid is selected from the group consisting of avipox, orthopox, and suipox nucleic acid.

30 9. The poxviral nucleic acid of claim 7, wherein said nucleic acid is selected from the group consisting of vaccinia, fowlpox, canary pox and swinepox.

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10. The poxviral nucleic acid of claim 7, wherein said nucleic acid is selected from the group consisting of TROVAC, NYVAC, ALVAC, MVA, Wyeth, and Poxvac-TC.
- 5        11. The nucleic acid of any one of claims 3 through 10 further comprising a nucleic acid sequence encoding at least one member selected from the group comprising cytokines, lymphokines, and co-stimulatory molecules.
- 10      12. A vector comprising the nucleic acid of any one of claims 3 through 11.
13. The vector of claim 12, wherein said vector is selected from the group consisting of recombinant viruses and bacteria.
14. The recombinant virus vector of claim 13, selected from the group consisting of adenovirus, alphavirus and poxvirus.
- 15      15. The poxvirus of claim 14, selected from the group consisting of avipox, orthopox, and suipox.
- 20      16. The poxvirus of claim 14, selected from the group consisting of vaccinia, fowlpox, canary pox, and swinepox.
17. The poxvirus of claim 14, selected from the group consisting of TROVAC, NYVAC, ALVAC, MVA, Wyeth, and Poxvac-TC.
- 25      18. The vector of any one of claims 12 through 17 further comprising a nucleic acid sequence encoding at least one member selected from the group consisting of cytokines, lymphokines, and co-stimulatory molecules.
- 30      19. A cell comprising a nucleic acid according to any one of claims 3 through 11 wherein the cell expresses a CEA agonist polypeptide.

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20. The cell of claim 19 wherein the cell additionally expresses a MHC HLA class I molecule.

21. The cell of claim 19 wherein the cell is an antigen-presenting cell.

5

22. The cell of claim 21 wherein the cell is a dendritic cell.

10 23. A mixture comprising a nucleic acid according to any one of claims 3 through 11 and at least one member selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor.

15 24. A mixture comprising a vector according to any one of claims 12 through 18 and at least one member selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor.

20 25. A mixture comprising a CEA agonist polypeptide according to any one of claims 1 or 2 and at least one member selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor.

25 26. A mixture comprising a cell according to any one of claims 19 through 22 and at least one member selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor.

30 27. A pharmaceutical composition comprising a CEA agonist polypeptide according to any one of claims 1 or 2, and a pharmaceutically acceptable diluent or carrier.

28. A pharmaceutical composition comprising a nucleic acid according to any one

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of claims 3 through 11, and a pharmaceutically acceptable diluent or carrier.

29. A pharmaceutical composition comprising a vector according to any one of  
claims

5 12 through 18, and a pharmaceutically acceptable diluent or carrier.

30. A pharmaceutical composition comprising a cell according to any one of  
claims 19 through 22, and a pharmaceutically acceptable diluent or carrier.

10 31. A pharmaceutical composition comprising a mixture according to any one of  
claims

23 through 26, and a pharmaceutically acceptable diluent or carrier.

32. The pharmaceutical composition of any one of claims 27 through 31 further  
15 comprising an adjuvant.

33. A method of inducing an immune response in an animal directed against a  
member selected from the group consisting of:

a CEA protein or fragment thereof,

20 a CEA agonist polypeptide according to any one of claims 1 or 2,

a CEA epitope,

a modified CEA epitope,

cells expressing a CEA protein or fragment thereof, CEA agonist

polypeptide according to any one of claims 1 or 2, CEA epitope,

25 modified CEA epitope, and

cells binding a CEA protein or fragment thereof, CEA agonist

polypeptide according to any one of claims 1 or 2, CEA epitope,

modified CEA epitope

comprising administering to said animal a CEA agonist polypeptide or

30 fragment thereof according to any one of claims 1 or 2 in an amount sufficient  
to induce an immune response.

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34. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:

a CEA protein or fragment thereof,

a CEA agonist polypeptide according to any one of claims 1 or 2,

5 a CEA epitope,

cells expressing a CEA protein or fragment thereof, CEA agonist

polypeptide according to any one of claims 1 or 2, CEA epitope,

modified CEA epitope, and

cells binding a CEA protein or fragment thereof, CEA agonist

10 polypeptide according to any one of claims 1 or 2, CEA epitope,  
modified CEA epitope

comprising administering to said animal a nucleic acid according to any one of claims 3 through 11 in an amount sufficient to induce an immune response.

15 35. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:

a CEA protein or fragment thereof,

a CEA agonist polypeptide according to any one of claims 1 or 2,

a CEA epitope,

20 a modified CEA epitope,

cells expressing a CEA protein or fragment thereof, CEA agonist

polypeptide according to any one of claims 1 or 2, CEA epitope,

modified CEA epitope, and

cells binding a CEA protein or fragment thereof, CEA agonist

25 polypeptide according to any one of claims 1 or 2, CEA epitope,  
modified CEA epitope

comprising administering to said animal a vector according to any one of claims 12 through 18 in an amount sufficient to induce an immune response.

30 36. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:

a CEA protein or fragment thereof,

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a CEA agonist polypeptide according to any one of claims 1 or 2,  
a CEA epitope,  
cells expressing a CEA protein or fragment thereof, CEA agonist  
polypeptide according to any one of claims 1 or 2, CEA epitope,  
5 modified CEA epitope, and  
cells binding a CEA protein or fragment thereof, CEA agonist  
polypeptide according to any one of claims 1 or 2, CEA epitope,  
modified CEA epitope  
comprising administering to said animal a cell according to any one of claims  
10 19 through 22 in an amount sufficient to induce an immune response.

37. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:  
15 a CEA protein or fragment thereof,  
a CEA agonist polypeptide according to any one of claims 1 or 2,  
a CEA epitope,  
a modified CEA epitope,  
cells expressing a CEA protein or fragment thereof, CEA agonist  
polypeptide according to any one of claims 1 or 2, CEA epitope,  
20 modified CEA epitope, and  
cells binding a CEA protein or fragment thereof, CEA agonist  
polypeptide  
according to any one of claims 1 or 2, CEA epitope, modified  
CEA epitope  
25 comprising administering to said animal a mixture according to any one of claims 23 through 26 in an amount sufficient to induce an immune response.

38. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:  
30 a CEA protein or fragment thereof,  
a CEA agonist polypeptide according to any one of claims 1 or 2,  
a CEA epitope,

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cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope, and

cells binding a CEA protein or fragment thereof, CEA agonist

5 polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope

comprising administering to said animal a composition according to any one of claims 27 through 32 in an amount sufficient to induce an immune response.

10

39. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the CEA agonist polypeptide according to any one of claims 1 or 2.

15

40. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the nucleic acid according to any one of claims 3 through 11.

20

41. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an, effective amount of the vector according to any one of claims 12 through 18.

25

42. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the cell according to any one of claims 19 through 22.

43. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the mixture according to any one of claims 23 through 26.

30

44. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the

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composition according to any one of claims 27 through 32.

45. The methods of any one of claims 39 through 44 wherein the carcinoma cell is  
a gastrointestinal, breast, pancreatic, bladder, ovarian, lung or prostate  
5 carcinoma cell.

46. A treatment for cancer comprising any one of the methods of claims 39 to 45.

10 47. The use of a CEA agonist polypeptide of claim 1 for the manufacture of a  
medicament for the treatment of cancer.

48. The use of claim 47, wherein said polypeptide comprises the amino acid  
sequence of SEQ. ID NO. 1.

15 49. The use of an isolated, purified or recombinant nucleic acid sequence having  
the sequence of SEQ. ID NO. 2 for the manufacture of a medicament for the  
treatment of cancer.

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ATGGAGTCTCCCTCGGCCCCCTCCCCACAGATGGTCATCCCTGGCAGAGGCTCCTGCTC -  
 1 TACCTCAGAGGGAGCCGGGGAGGGGTGTCTACCACGTAGGGGACCGTCTCCGAGGACGAG 60

a M E S P S A P P H R R W C I P W Q R L L L -  
 ACAGCCTCACTTCTAACCTCTGGAACCCGCCACACTGCCAAGCTCACTATTGAATCC 120  
 61 TGTCGGAGTGAAGATTGGAAAGACCTTGGCGGGTGGTGACGGTTCGAGTGATAACTTAGG

a T A S L L T F W N P P T T A K L T I E S -  
 ACGCCGTTCAATGTCGAGAGGGGAAGGAGGTGCTTCTACTTGTCCACAATCTGGCCAG 180  
 121 TGCGGCAAGTTACAGCGTCTCCCTCCTCACGAAGATGACAGGTGTTAGACGGGTC

a T P F N V A E G K E V L L L V H N L P Q -  
 CATCTTTTGGCTACAGCTGGTACAAAGGTGAAAGAGTGGATGGCAACCGTCAAATTATA 240  
 181 GTAGAAAAACCGATGTCGACCATGTTCCACTTCTCACCTACCGTTGGCAGTTAATAT

a H L F G Y S W Y K G E R V D G N R Q I I -  
 GGATATGTAATAGGAACCTAACAAAGCTACCCCGGGCCATACAGTGGTCGAGAGATA 300  
 241 CCTATACATTATCCTTGAGTTGTCGATGGGTCCCGGGGTATGTCACCAAGCTCTAT

a G Y V I G T Q Q A T P G P A Y S G R E I -  
 ATATACCCCAATGCATCCCTGCTGATCCAGAACATCATCAGAACATGACACAGGATTCTAC 360  
 301 TATATGGGGTTACGTAGGGACGACTAGGTCTTGTAGTAGGTCTTACTGTGTCCTAAAGATG

a I Y P N A S L L I Q N I I Q N D T G F Y -  
 ACCCTACACGTCAAAAGTCAGATCTTGTGAATGAAGAAGCAACTGGCCAGTTCCGGGT 420  
 361 TGGGATGTCGAGTATTCAGTCAGAACACTTACTTCTCGTTGACGGTCAAGGCCAT

a T L H V I K S D L V N E E A T G Q F R V -  
 TACCCGGAGCTGCCAACGCCCTCCATCTCAGCAACAACTCCAAACCGTGGAGGACAAG 480  
 421 ATGGGCCTCGACGGGTTGGGAGGTAGAGGTGTTGAGGTTGGCACCTCCTGTT

a Y P E L P K P S I S S N N S K P V E D K -  
 GATGCTGTGGCCTTCACCTGTGAACCTGAGACTCAGGACGCAACCTACCTGTGGTGGTA 540  
 481 CTACGACACCGGAAGTGGACACTTGGACTCTGAGTCCTCGTGGATGGACACCACCCAT

a D A V A F T C E P E T Q D A T Y L W W V -  
 AACAAATCAGAGCCTCCGGTCAGTCCCAGGCTGCAGCTGCTCAATGGCAACAGGACCC 600  
 541 TTGGTAGTCTCGAGGGCCAGTCAGGGTCCGACGTCGACAGGTTACGGTTGTCCTGGGAG

a N N Q S L P V S P R L Q L S N G N R T L -  
 ACTCTATTCAATGTCACAAGAAATGACACAGCAAGCTACAATGTGAAACCCAGAACCCA 660  
 601 TGAGATAAGTTACAGTGTCTTACTGTGTCGTTGATGTTACACTTGGGTCTGGGT

a T L F N V T R N D T A S Y K C E T Q N P -  
 GTGAGTGGCAGGGCCAGTGATTCAGTCATCTGAATGTCTCTATGGCCCGGATGGGG 720  
 661 CACTCACGGTCCCGTCACTAAGTCAGTAGGACTTACAGGAGATAACGGGCTACGGGG

a V S A R R S D S V I L N V L Y G P D A P -

Figure 1(A)

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ACCATTTCCCTCTAAACACATCTTACAGATCAGGGAAAATCTGAACCTCTCCGCCAC  
721 TGGTAAAGGGGAGATTGTGAGATGTCTAGTCCCCTTTAGACTTGGAGAGGACGGTG 780

T I S P L N T S Y R S G E N L N L S C H -  
GCAGCCTCTAACCCACCTGCACAGTACTCTGGTTTGTCAATGGACTTTCCAGCAATCC  
781 CGTCGGAGATTGGGTGGACGTGTCAAGAACCAAACAGTTACCCCTGAAAGGTCGTTAGG 840

A A S N P P A Q Y S W F V N G T F Q Q S -  
ACCCAAGAGCTCTTATCCCCAACATCACTGTGAATAATAGGGATCCTATACGTGCCAA  
841 TCGGTTCTCGAGAAATAGGGTTGTAGTGACACTTATTATCACCTAGGATATGCACGGTT 900

T Q E L F I P N I T V N N S G S Y T C Q -  
GCCCATAACTCAGACACTGGCCTCAATAGGACCACAGTCACGACGATCACAGTCTATGAG  
901 CGGGTATTGAGTCTGTGACCGGAGTTATCCTGGTCACTGTGCTGAGTGTCAAGATACTC 960

A H N S D T G L N R T T V T T I T V Y E -  
CCACCCAAACCCCTCATCACCAAGCAACAACCTCAACCCCGTGGAGGATGAGGATGCTGTA  
961 GGTGGGTTGGAAAGTAGTGGTCGTTGGAGGTTGGGCACCTCTACTCCTACGACAT 1020

P P K P F I T S N N S N P V E D E D A V -  
GCCTTAACCTGTGAACCTGAGATTCAAACACAAACCTACCTGTGGTGGTAAATAATCAG  
1021 CGGAATTGGACACTTGGACTCTAAGTCTTGTGTTGGATGGACACCACCCATTATTAGTC 1080

A L T C E P E I Q N T T Y L N W W V N N Q -  
AGCCTCCCGTCAGTCCCAGGCTGCAGCTGTCAATGACAACAGGACCCCACTCTACTC  
1081 TCGGAGGGCCAGTCAGGGTCCGACGTCGACAGGTTACTGTGCTGGAGTGGAGATGAG 1140

S L P V S P R L Q L S N D N R T L T L L -  
AGTGTACAAGGAATGATGTAGGACCCATGAGTGTGGAATCCAGAACGAAATTAAAGTGT  
1141 TCACAGTGTCTTACTACATCCTGGATACTCACACCTTAGGTCITGCTTAATTACAAA 1200

S V T R N D V G P Y E C G I Q N E L S V -  
GACCACAGCGACCCAGTCATCCTGAATGTCCCTATGGCCCAGACGACCCACCAATTCC  
1201 CTGGTGTGGCTGGTCAGTAGGACTTACAGGAGATACCGGCTGTGCTGGGTGTTAAAGG 1260

D H S D P V I L N V L Y G P D D P T I S -  
CCCTCATACACCTATTACCGTCCAGGGTGAACCTCAGCCTCTCCGCCATGCAGCCTCT  
1261 GGGAGTATGTGGATAATGGCAGGTCCCACTTGGAGTGGAGAGGACGGTACGTCGGAGA 1320

P S Y T Y Y R P G V N L S L S C H A A S -  
AACCCACCTGCACAGTATTCTTGGCTGATTGATGGAAACATCCAGCAACACACAAAGAG  
1321 TTGGTGGACGTGTCAAGAACCGACTAACTACCCCTGTAGGTCGTTGTGTTCTC 1380

N P P A Q Y S W L I D G N I Q Q H T Q E -  
CTCTTATCTCCAAACATCACTGAGAAGAACAGCGGACTCTATACCTGCCAGGCCAAATAC  
1381 GAGAAATAGAGGTTGTAGTGACTCTCTGTGCGCTGAGATATGGACGGTCCGGTTATTG 1440

L F I S N I T E K N S G L Y T C Q A N N -

**Figure 1(B)**

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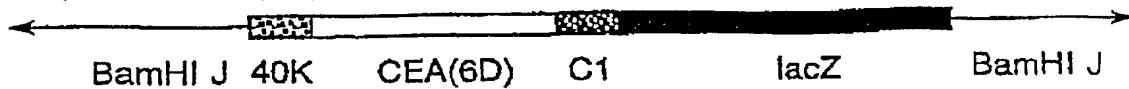
TCAAGCCAGTGGCCACAGCAGGACTACAGTCAGAACATCACAGTCTCTGCAGGAGCTGCC  
 1441 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1500  
 AGTCGGTCACCGGTGCGTCTGATGTCAGTTCTGTTAGTGTCAAGAGACGCCCTGACGGG  
  
 a S A S G H S R T T V K T I T V S A E L P -  
  
 a AAGCCCTCCATCTCCAGCAACAACCTCAAACCGTGGAGGACAGGATGCTGTGGCCCTC  
 1501 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1560  
 TTCGGGAGGTAGAGGTGCTTGAGGTTGGGACCTCCCTGTCACGACACCGGAAG  
  
 a K P S I S S N N S K P V E D K D A V A F -  
  
 a ACCTGTGAACCTGAGGCTCAGAACACAAACCTACCTGTGGTGGTAAATGGTCAGAGCCTC  
 1561 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1620  
 TGGACACTTGGACTCCGAGTCCTGTGTTGGATGGACACCACCCATTACAGTCCTGGAG  
  
 a T C E P E A Q N T T Y L W W V N G Q S L -  
  
 a CCAGTCAGTCCCAGGCTGCAGCTGCCAATGGCAACAGGACCCCTCACTCTATTCAATGTC  
 1621 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1680  
 GGTCACTCAGGGTCCGACGTCGACAGGTTACCGTGTCTGGAGTGAGATAAGTTACAG  
  
 a P V S P R L Q L S N G N R T L T L F N V -  
  
 a ACAAGAAATGACGCAAGAGCCTATGTATGTTGAATCCAGAACTCAGTGAGTGCAACCGC  
 1681 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1740  
 TGTTCTTACTCGTTCTCGGATAACATACACCTTAGGTCTTGAGTCACTCAGTTGGCG  
  
 a T R N D A R A Y V C G I Q N S V S A N R -  
  
 a AGTGACCCAGTCACCCCTGGATGTCTCTATGGGCCGACACCCCCCATCATTTCCCCCCC  
 1741 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1800  
 TCACTGGGTCACTGGGACCTACAGGAGATAACCCGCTGTGGGGTAGTAAAGGGGGGT  
  
 a S D P V T L D V L Y G P D T P I I S P P -  
  
 a GACTCGCTTACCTTTCGGGAGCGGACCTCAACCTCTCTGCCACTCGGCCCTCATACCCA  
 1801 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1860  
 CTGAGCAGAATGGAAAGCCCTCGCTGGAGTTGGAGAGGACGGTGAGCCGGAGATTGGGT  
  
 a D S S Y L S G A D L N L S C H S A S N P -  
  
 a TCCCCCGCAGTATTCTGGCGTATCAATGGATAACCGCAGCAACACACACAAAGTTCTCTT  
  
 a 1861 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1920  
 AGGGGCGTCATAAGAACCGCATAGTTACCCATGGCGTGTGTGTGTTCAAGAGAAA  
  
 a S P Q Y S W R I N G I P Q Q H T Q V L F -  
  
 a ATGGCCAAAATCACGCCAAATAATAACGGGACCTATGCCCTTTGTCTCTAACCTGGCT  
 1921 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1980  
 TAGCGGTTTAGTGGGTTTATTATGGCCCTGGATAACGGACAAAACAGAGATTGAACCGA  
  
 a I A K I T P N N N G T Y A C F V S N L A -  
  
 a ACTGGCCGCAATAATTCCATAGTCAGAGACATCACAGTCTGCATCTGGAACTTCTCCT  
 1981 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2040  
 TGACCGGCCTTAATAAGGTATCAGTTCTCGTAGTGTCAAGAGACGTAGACCTTGAAGAGGA  
  
 a T G R N N S I V K S I T V S A S G T S P -  
  
 a GGTCTCTCAGCTGGGCCACTGTGGCATCATGATTGGAGTGTGGTGGGGTTGCTCTG  
 2041 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2100  
 CCAGAGAGTCGACCCGGTGACAGCGTAGTACTAACCTCACGACCAACCCCAACGAGAC  
  
 a G L S A G A T V G I M I G V L V G V A L -  
  
 a ATATAG ← SEQ ID NO: 2  
 2101 ----- 2106  
 TATATC  
  
 a I \* ← SEQ ID NO: 1.

**Figure 1(C)**

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## Genomic Structure of Recombinant Poxviruses Expressing CEA(6D)

A. Fowlpox rF-CEA(6D) vT233



B. Vaccinia rV-CEA(6D) vT237



C. MVA rMVA-CEA(6D)

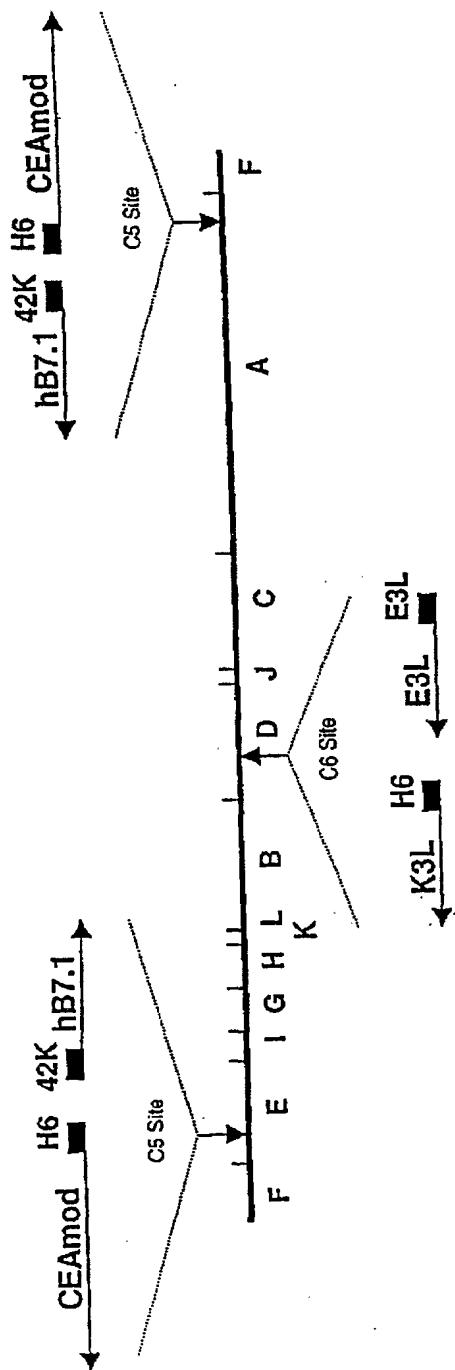


BamHI J is the site of insertion in the fowlpox genome of the foreign genes.  
 Hind III J is the site of insertion in the vaccinia virus genome.  
 Deletion III is the site of insertion in the MVA genome.  
 40K, C1, P1 and P2 are poxviral promoters.

*(not drawn to scale)*

**Figure 2**

**ALVAC(2)-CEAmod/hB7.1 (vCP1586)**  
**(ALVAC Xhol Restriction Map)**



**Figure 3**

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**Sequence of the H6-promoted human  
CEAmod/42K-promoted B7.1 insertion cassette**

|  |     |     |     |     |     |
|--|-----|-----|-----|-----|-----|
| 10   | 20  | 30  | 40  | 50  | 60  |
| GCCCTTCGCT CTCGCGCGTT TCGGTGATGA CGGTGAAAAC CTCTGACACA TGCAGCTCCC  |     |     |     |     |     |
| CGGGAAAGCA GAGCGCGCAA AGCCACTACT GCCACTTTG GAGACTGTGT ACGTCGAGGG   |     |     |     |     |     |
| 70   | 80  | 90  | 100 | 110 | 120 |
| GGAGACGGTC ACAGCTTGTC TGTAAGCGGA TGCCGGGAGC AGACAAGCCC GTCAGGGCGC  |     |     |     |     |     |
| CCTCTGCCAG TGTGAAACAG ACATTGCGCT ACGGCCCTCG TCTGTTCGGG CAGTCCCAG   |     |     |     |     |     |
| 130  | 140 | 150 | 160 | 170 | 180 |
| GTCAGCGGGT GTTGGCGGTG CTGCGGGCTG GCTTAACATAT CGGGCATCAG ACCAGATTTG |     |     |     |     |     |
| CAGTCGCCCA CAACCGCCCA CAGCCCCGAC CGAATTGATA CGCCGTAGTC TCGTCTAAC   |     |     |     |     |     |
| 190  | 200 | 210 | 220 | 230 | 240 |
| ACTGAGAGTG CACCATATGC GGTGTGAAAT ACCGCACAGA TGCGTAAGGA GAAAATACCG  |     |     |     |     |     |
| TGACTCTCAC GTGGTATACG CCACACTTTA TGGCGTGTCT ACGCATTCCCT CTTTATGGC  |     |     |     |     |     |
| 250  | 260 | 270 | 280 | 290 | 300 |
| CATCAGGGCG CATTGCGCAT TCAGGCTGCG CAACTGTTGG GAAGGGCGAT CGGTGCGGGC  |     |     |     |     |     |
| GTAGTCCCGC GTAAGCGGTA AGTCCGACCG GTTGACAACC CTTCGGCTA CCCACGCCCG   |     |     |     |     |     |
| 310  | 320 | 330 | 340 | 350 | 360 |
| CTCTTCGCTA TTACGCCAGC TGGCGAAAGG GGGATGTGCT GCAAGGGCGAT TAAGTTGGGT |     |     |     |     |     |
| GAGAAGCGAT AATGCGGTGCG ACCGCTTTC CCCTACACGA CGTTCCGCTA ATTCAACCCA  |     |     |     |     |     |
| 370  | 380 | 390 | 400 | 410 | 420 |
| AACGCCAGGG TTTTCCCAGT CACGACGTG TAAAACGACG GCCAGTGCCA AGCTTGGCTG   |     |     |     |     |     |
| TTGCGGTCCC AAAAGGGTCA GTGCTGCAAC ATTTTGCTGC CGGTACACGGT TCGAACCGAC |     |     |     |     |     |
| 430  | 440 | 450 | 460 | 470 | 480 |
| CAGGTATTCCT AAACTAGGAA TAGATGAAAT TATGTGCAAA GGAGATACCT TTAGATATGG |     |     |     |     |     |
| GTCCATAAGA TTGATCCTT ATCTACTTTA ATACACGTTT CCTCTATGGA AATCTATACC   |     |     |     |     |     |
| <u>c</u> ALVAC'S C5 LOCUS LEFT FLANKING ARM <u>c</u> >             |     |     |     |     |     |
| 490  | 500 | 510 | 520 | 530 | 540 |
| ATCTGATTTA TTGGTTTTT CATAATCATA ATCTAACAAAC ATTTTCACTA TACTATACCT  |     |     |     |     |     |
| TAGACTAAAT AAACAAAAAA GTATTAGTAT TAGATTGTTG TAAAAGTGAT ATGATATGGA  |     |     |     |     |     |
| <u>c</u> ALVAC'S C5 LOCUS LEFT FLANKING ARM <u>c</u> >             |     |     |     |     |     |
| 550  | 560 | 570 | 580 | 590 | 600 |
| TCTTGACAAA GTGCCATTA GTAGTATAGA CTTATACCTT GAAACCATAG TATACTTTAG   |     |     |     |     |     |
| AGAACGTGTT CAGCGGTAAAT CATCATATCT GAATATGAAA CATTGGTATC ATATGAAATC |     |     |     |     |     |
| <u>c</u> ALVAC'S C5 LOCUS LEFT FLANKING ARM <u>c</u> >             |     |     |     |     |     |
| 610  | 620 | 630 | 640 | 650 | 660 |
| CGCGTCATCT TCTTCATCTA AAACAGATT ACAACAATAA TCATCGTCGT CATCTTCATC   |     |     |     |     |     |
| GCGCAGTAGA AGAAGTAGAT TTGCTAA TGTGTTATT AGTAGCAGCA GTAGAAGTAG      |     |     |     |     |     |
| <u>c</u> ALVAC'S C5 LOCUS LEFT FLANKING ARM <u>c</u> >             |     |     |     |     |     |
| 670  | 680 | 690 | 700 | 710 | 720 |
| TTCTTAAAG TTTCATATT CAATAACTTT CTTTCTAAA ACATCATCTG AATCAATAAA     |     |     |     |     |     |
| AAGTAATTTC AAAAGTATAA GTTATTGAAA GAAAAGATTG TGTAGTAGAC TTAGTTATT   |     |     |     |     |     |
| <u>c</u> ALVAC'S C5 LOCUS LEFT FLANKING ARM <u>c</u> >             |     |     |     |     |     |
| 730  | 740 | 750 | 760 | 770 | 780 |
| CATAGAACCG TATAGAGCGT TAATCTCCAT TGTAAAATAT ACTAACCGGT TGCTCATGAT  |     |     |     |     |     |

Figure 4(A)

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**Sequence of the H6-promoted human  
CEAmod/42K-promoted B7.1 insertion cassette (Cont.)**

```

GTATCTGCC ATATCTCGCA ATTAGAGGTA ACATTTATA TGATTGCGCA ACGAGTACTA
C ALVAC'S C5 LOCUS LEFT FLANKING ARM C >

790      800      810      820      830      840
GTACTTTTT TCATTATTTA GAAATTATGC ATTTTAGATC TTATAAGCG GCCGTGATTA
CATGAAAAAA AGTAATAAT CTTAATACG TAAAATCTAG AAATATTCGC CGGCACTAAT
C ALVAC'S C5 LOCUS LEFT FLANKING ARM C >

850      860      870      880      890      900
ACTAGTCATA AAAACCCGGG ATCGATTCTA GACTCGACCT CGAACGTTGC ATGCCGGTGC
TGATCAGTAT TTTGGGGCCC TAGCTAAGAT CTGAGCTGGA GCTTCGAACG TACGGCCAGC

910      920      930      940      950      960
AGCTATATCA GAGCAACCCC AACCAGCACT CCAATCATGA TGCCGACAGT GGCCCCAGCT
TCGATATAGT CTCGTTGGGG TTGGTGTGA CGTTAGTACT ACGGCTGTCA CGGGGGTCGA
< a MODIFIED CEA ORF. a >

970      980      990      1000      1010      1020
GAGAGACCAG GAGAAAGTTCC AGATGCAGAG ACTGTGATGC TCTTGACTAT GGAATTATTG
CTCTCTGGTC CTCTPTCAAGG TCTACGTCTC TGACACTACG AGAACTGATA CCTTAATAAC
< a MODIFIED CEA ORF. a >

1030     1040     1050     1060     1070     1080
CGGCCAGTAG CCAAGTTAGA GACAAAAACAG GCATAGGTCC CGTTATTATT TGGCGTGATT
GCCGGTCATC GGTTCAATCT CTGTTTGTC CGTATCCAGG GCAATATAAA ACCGCACTAA
< a MODIFIED CEA ORF. a >

1090     1100     1110     1120     1130     1140
TTGGCGATAA AGAGAACTTG TGTGTGTTGC TGCCTGATCC CATTGATACG CCAAGAAATAC
AACCCTTATT TCTCTTGAAC ACACACAACG ACGCCATAGG GTAACATATGC GGTTCTTATG
< a MODIFIED CEA ORF. a >

1150     1160     1170     1180     1190     1200
TGCGGGGATG GGTTAGAGGC CGAGTGGCAG GAGAGGTGA GGTCCGCTCC CGAAAGGTAA
ACGGCCCTAC CCAATCTCCG GCTCACCGTC CTCTCCAAT CCAGGGGAGG GCTTTCCATT
< a MODIFIED CEA ORF. a >

1210     1220     1230     1240     1250     1260
GACGACTCTG GGGGGAAAT GATGGGGGTG TCCGGCCAT AGAGGACATC CAGGGTGACT
CTGCTCAGAC CCCCTTTTA CTACCCCCAC AGGCGGGGTAG TCTCCTGTAG GTCCCACGTGA
< a MODIFIED CEA ORF. a >

1270     1280     1290     1300     1310     1320
GGGTCACTGC GGTTTGCACT CACTGAGTTG TGGATTCCAC ATACATAGGC TCTTGCCTCA
CCCAGTGACG CCAAACGTGA GTGACTCAAG ACCTAAGGTG TATGTATCCG AGAACGCAGT
< a MODIFIED CEA ORF. a >

1330     1340     1350     1360     1370     1380
TTTCTGTGA CATTGAATAG AGTGAGGGTC CTGTTGCCAT TGGACAGCTG CAGCCTGGGA
AAAGAACACT GAACTTATC TCACTCCCCAG GACAACGTTA ACCTGTGAC GTCGGACCCCT
< a MODIFIED CEA ORF. a >

1390     1400     1410     1420     1430     1440
CTGACTGGGA GGCTCTGACC ATTTACCCAC CACAGGTAGG TTGTGTTCTG AGCCTCAGGT
GACTGACCCCT CCGAGACTGG TAAATGGGTG GTGTCCATCC AACACAAGAC TCGGAGTCCA
< a MODIFIED CEA ORF. a >

```

Figure 4(B)

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**Sequence of the H6-promoted human  
CEAmod/42K-promoted B7.1 insertion cassette (Cont.)**

1450 1460 1470 1480 1490 1500  
 TCACAGGTGA AGGCCACAGC ATCCTTGTCC TCCACGGGTG TTGGAGTTGTT GCTGGAGATG  
 AGTGTCCACT TCCGGTGTG TAGGAACAGG AGGTGCCCCA ACCTCAACAA CGACCTCTAC  
 < a a MODIFIED CEA ORF. a a  
  
 1510 1520 1530 1540 1550 1560  
 GAGGGCTGG GCAGCTCCGC AGAGACTGTG ATTGTCTTGA CTGTAGTCCT GCTGTGGCCA  
 CTCCCGAACCG CGTCGAGGCG TCTCTGACAC TAACAGAACT GACATCAGGA CGACACCGGT  
 < a a MODIFIED CEA ORF. a a  
  
 1570 1580 1590 1600 1610 1620  
 CTGGCTGAGT TATTGGCTTG GCAGGTATAG AGTCCCGTGT TCTTCTCAGT GATGTGGAG  
 GACCGACTCA ATAACCGGAC CGTCCATATC TCAGGCGACA AGAAGAGTCA CTACAACTC  
 < a a MODIFIED CEA ORF. a a  
  
 1630 1640 1650 1660 1670 1680  
 ATAAAGAGCT CTTGTGTGTG TTGCTGGATG TTCCCATCAA TCAGCCAAGA ATACTGTGCA  
 TATTTCTCGA GAACACACAC AACGACCTAC AAGGGTAGTT AGTCGGTTCT TATGACACGT  
 < a a MODIFIED CEA ORF. a a  
  
 1690 1700 1710 1720 1730 1740  
 GGTGGTTAG AGGCTGCATG GCAGGGAGGG CTGAGGTCTCA CCCCTGGACG GTAAATAGGTG  
 CCACCCAATC TCCGACGTAC CGTCCCTCTCC GACTCCAAGT GGGGACCTGC CATTATCCAC  
 < a a MODIFIED CEA ORF. a a  
  
 1750 1760 1770 1780 1790 1800  
 TATGAGGGGG AAATGGTGGG GTCGTCTGGG CCATAGAGGA CATTCAAGGAT GACTGGGTGCG  
 ATACTCCCCC TTACCAACCC CAGCAGACCC GGTTATCTCCT GTAAAGTCCTA CTGACCCAGC  
 < a a MODIFIED CEA ORF. a a  
  
 1810 1820 1830 1840 1850 1860  
 CTGTGGTCAA CACTTAATTG GTTCTGGATT CCACACTCAT AGGGTCTTAC ATCATTCTT  
 GACACCAGTT GTGAATTAAG CAAGACCTAA GGTGTGAGTA TCCCAGGATG TAGTAAGGAA  
 < a a MODIFIED CEA ORF. a a  
  
 1870 1880 1890 1900 1910 1920  
 GTGACACTGA GTAGAGTGAG GGTCTGTGTC TCATTGGACCA GCTGCAGCCT GGGACTGACCC  
 CACTGTGACT CATCTCACTC CCAGGACAAAC AGTAACCTGT CGACGTCGGA CCCTGACTGG  
 < a a MODIFIED CEA ORF. a a  
  
 1930 1940 1950 1960 1970 1980  
 GGGAGGCTCT GATTATTTAC CCACCACAGG TAGGTTGTGT TCTGAATCTC AGGTTCACAG  
 CCCTCCGAGA CTAATAAAATG GGTGGTGTCC ATCCAACACA AGACTTAGAG TCCAAGTGTG  
 < a a MODIFIED CEA ORF. a a  
  
 1990 2000 2010 2020 2030 2040  
 GTTAAGGCTA CAGCATCCTC ATCCTCCACG GGGTTGGAGT TGTTGCTGGT GATGAAGGGT  
 CAATTCCGAT GTCGTAGGAG TAGGAGGTGC CCCAACCTCA ACAACGACCA CTACTTCCCA  
 < a a MODIFIED CEA ORF. a a  
  
 2050 2060 2070 2080 2090 2100  
 TTGGGGTGGCT CATAGACTGT GATCGTGTG ACTGTGGTCC TATTGAGGCC AGTGTCTGAG  
 AACCCACCGA GTATCTGACA CTAGCAGCAC TGACACCAGG ATAACCTCCGG TCACAGACTC  
 < a a MODIFIED CEA ORF. a a  
  
 2110 2120 2130 2140 2150 2160  
 TTATGGGCTT CGCACGTTATA GGATCCACTA TTATTGACAG TGATGGGG GATAAAAGAGC

**Figure 4(C)**

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**Sequence of the H6-promoted human  
CEAmod/42K-promoted B7.1 insertion cassette (Cont.)**

AATAACCCGAA CCGTGCATAT CCTAGGTGAT AATAAGTGTC ACTACAACCC CTATTTCTCG  
 < a MODIFIED CEA ORF. a a  
 2170 2180 2190 2200 2210 2220  
 TCTTGGGTGG ATTGCTGGAA AGTCCCATTG ACAAAACCAAG AGTACTGTGTC AGGTGGGTTA  
 AGAACCCACC TAACGGACCTT TCAGGGTAAC TGTTTGGGTTTC TCATGACACG TCCACCCAAT  
 < a a MODIFIED CEA ORF. a a  
 2230 2240 2250 2260 2270 2280  
 GAGGCTGCGT GGGCAGGAGAG GTTCAGAATT TCCCCCTGATC TGTAAGATGT GTTGTAGAGGG  
 CTCCGACGCA CCGTCCTCTC CAAGTCTAAA AGGGGACTAG ACATTCTACA CAAATCTCCC  
 < a a MODIFIED CEA ORF. a a  
 2290 2300 2310 2320 2330 2340  
 GAAATGGTGG GGGCATCCGG GCCATAGAGG ACATTCAGGA TGACTGAATC ACTGCCGCTG  
 CTTTACCAACC CCCGTAGGCC CGGTATCTCC TGTAAGTCT ACTGACTTAG TGACCGGGAC  
 < a a MODIFIED CEA ORF. a a  
 2350 2360 2370 2380 2390 2400  
 GCACTCACIG GGTCTGGGT TTACATMMG TAGCTTGCTG TGTCATTCT TGIGACATTG  
 CGTGAGTGAC CCAAGACCCA AAGTGTAAAC ATCGAACGAC ACAGTAAAGA ACACTGTAAAC  
 < a a MODIFIED CEA ORF. a a  
 2410 2420 2430 2440 2450 2460  
 AATAGAGTGA GGGTCTGTGTT GCCTTGGAC AGCTGCAGCC TGGGACTGAC CGGGAGGCTC  
 TTATCTCACT CCCAGGACAA CGGTAACTG TCGACGTCGG ACCCTGACTG GGCCTCCGAG  
 < a a MODIFIED CEA ORF. a a  
 2470 2480 2490 2500 2510 2520  
 TGATTTGTITA CCCACCCACAG GTAGGTTGGG TCCTGAGTCT CAGGTTCACCA GGTGAAGGCG  
 ACTAACAAAT GGGTGGTGTG CATCCAAACGC AGGACTCAGA GTCCAAGTGT CCACTTCCGG  
 < a a MODIFIED CEA ORF. a a  
 2530 2540 2550 2560 2570 2580  
 ACAGCATCCT TGTCTCTCAC GGGTTTGGAG TTGTTGCTG AGATGGAGGG CTGGGCAGC  
 TGTCGTAGGA ACAGGAGGTG CCCAAACCTC AACAAACGACC TCTACCTCCC GAACCCGTCG  
 < a a MODIFIED CEA ORF. a a  
 2590 2600 2610 2620 2630 2640  
 TCCGGGTATA CCCGGAACGT GCCAGTTGCT TCCTCATTCA CAAGATCTGA CTTTATGACG  
 AGCCCCATAT GGGCTTGTGAC CGGTCAACGA AGAAGTAAGT GTTCTAGACT GAAATACTGCG  
 < a a MODIFIED CEA ORF. a a  
 2650 2660 2670 2680 2690 2700  
 TGTAGGGTGT AGAATCTGT GTCAATTCTGG ATGATGTTCT GGATCAGCAG GGATGCATTG  
 ACATCCCACA TCTTAGGACA CAGTAAGACC TACTACAAGA CCTAGTCGTC CCTACGTAAC  
 < a a MODIFIED CEA ORF. a a  
 2710 2720 2730 2740 2750 2760  
 GGGTATATTA TCTCTCGACC ACTGTATGCG GGGCTGGGG TAGCTGTTG AGITCTATT  
 CCCATATAAT AGAGAGCTGG TGACATACGC CCGGGACCC ATCGAACAAAC TCAAGGATAA  
 < a a MODIFIED CEA ORF. a a  
 2770 2780 2790 2800 2810 2820  
 ACATATCCTA TAATTTGACG GTGGCATCC ACTCTTTCAC CTTTGATCCA GCTGTAGGCC  
 TGTATAGGAT ATTAACCTGC CAAACGGTAGG TGAGAAAGTG GAAACATGGT CGACATCGGT  
 < a a MODIFIED CEA ORF. a a

**Figure 4(D)**

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**Sequence of the H6-promoted human  
CEAmid/42K-promoted B7.1 insertion cassette (Cont.)**

|   |      |      |      |      |      |
|---|------|------|------|------|------|
| 2830  | 2840 | 2850 | 2860 | 2870 | 2880 |
| AAAAGATGCT GGGGAGATT GTGGACAAAGT AGAAGCACCT CCTTCCCTC TGCGACATTG    |      |      |      |      |      |
| TTTTCTACGA CCCCGCTAA CACCTGTTCA TCTTCGTGGA GGAAGGGAG ACGCTGTAAC     |      |      |      |      |      |
| < a a MODIFIED CEA ORF a a >  |      |      |      |      |      |
| 2890  | 2900 | 2910 | 2920 | 2930 | 2940 |
| AACGGCGTGG ATTCATAGT GAGCTGGCA GTGGTGGCG GGTTCCAGAA GGTTAGAAGT      |      |      |      |      |      |
| TTGCCGACCC TAAGTATCA CTGGAACCGT CACCAACCGC CCAAGGTCTT CCATCTTCA     |      |      |      |      |      |
| < a a MODIFIED CEA ORF a a >  |      |      |      |      |      |
| 2950  | 2960 | 2970 | 2980 | 2990 | 3000 |
| GAGGCTGTGA GCAGGAGCCT CTGCCAGGGG ATGCACCATC TGTGGGGAGG GGCGGAGGGA   |      |      |      |      |      |
| CTCCGACACT CGTCCTCGGA GACGGTCCCC TAGCTGGTAG ACACCCCTCC CGGGCTCCCT   |      |      |      |      |      |
| < a a MODIFIED CEA ORF a a >  |      |      |      |      |      |
| 3010  | 3020 | 3030 | 3040 | 3050 | 3060 |
| GACTCCATTA CGATAACAAAC TTAACGGATA TCGCGATAAT GAAATAATTT ATGATTATTT  |      |      |      |      |      |
| CTGAGGTAAT GCTATGTTTG AATTGCCTAT AGCGCTATTAA CTTTATTAAA TACTAATAAA  |      |      |      |      |      |
| < e e VACCINIA'S H6 PROMOTER e e >                                  |      |      |      |      |      |
| 3070  | 3080 | 3090 | 3100 | 3110 | 3120 |
| CTCGCTTCA ATTAAACACA ACCCTCAAGA ACCTTTGTAT TTATTTCAC TTTTTAAGTA     |      |      |      |      |      |
| GAGCGAAAGT AAATTGTGT TGGAGATTCT TGGAAACATA AATAAAAGTG AAAAATTCA     |      |      |      |      |      |
| < e e VACCINIA'S H6 PROMOTER e e >                                  |      |      |      |      |      |
| 3130  | 3140 | 3150 | 3160 | 3170 | 3180 |
| TAGAATAAAAG AAGCTCTAAT TANTAACGA GCAGATAGTC TCGTTCTCGC CCTGCTGAT    |      |      |      |      |      |
| ATCTTATTTTC TTCGAGGATTA ATTAATTGCT CGTCTATCAG AGCAAGAGCG GGACGGACTA |      |      |      |      |      |
| e >   |      |      |      |      |      |
| 3190  | 3200 | 3210 | 3220 | 3230 | 3240 |
| GACTAAATTA TTAACCCGGA TCGAATTCT TTATGGAA GAATATGATA ATATTTGGG       |      |      |      |      |      |
| CTGATTAATT AATTGGCCCT AGGTTAAGA AATAACCCCTT CTTATACTAT TATAAAACCC   |      |      |      |      |      |
| f 42K PROMOTER f >  |      |      |      |      |      |
| 3250  | 3260 | 3270 | 3280 | 3290 | 3300 |
| ATTTCAAAAT TGAATAATATA TAATTCAAT ATAAAATGGG CCACACACGG AGGCAGGGAA   |      |      |      |      |      |
| TAAAGTTTA ACTTTTATAT ATTAATGTTA TATTTTACCC GGTGTGIGCC TCCGCTCCCTT   |      |      |      |      |      |
| HUMAN B7.1 ORF >  |      |      |      |      |      |
| f 42K PROMOTER f >  |      |      |      |      |      |
| 3310  | 3320 | 3330 | 3340 | 3350 | 3360 |
| CATCACCATC CAAGTGTCCA TACCTCAATT TCTTTACGCT CTTGGTGTCTG GCTGGTCTTT  |      |      |      |      |      |
| GTAGTGGTAG GTTCACAGGT ATGGAGTTAA AGAAAGTCGA GAACCACGAC CGACCAAGAAA  |      |      |      |      |      |
| b b HUMAN B7.1 ORF b b >  |      |      |      |      |      |
| 3370  | 3380 | 3390 | 3400 | 3410 | 3420 |
| CTCACTCTG TTCAGGTGTT ATCCACCTGA CCAAGGAAGT GAAAGAAGTG GCAACGCTGT    |      |      |      |      |      |
| GAGTGAAGAC AAGTCCACAA TAGGTGCACT GGTTCCTTCA CTTTCTTCAC CGTTGCGACA   |      |      |      |      |      |
| b b HUMAN B7.1 ORF b b >  |      |      |      |      |      |
| 3430  | 3440 | 3450 | 3460 | 3470 | 3480 |
| CCTGTGGTCA CAATGTTCT GTTGAAGAGC TGGCACAAAC TCGCATCTAC TGGCAAAAGG    |      |      |      |      |      |
| GGACACCAGT GTTACAAAGA CAACTCTCG ACCGTGTTTG AGCGTAGATG ACCGTTTCC     |      |      |      |      |      |
| b b HUMAN B7.1 ORF b b >  |      |      |      |      |      |

Figure 4(E)

11/18

**Sequence of the H6-promoted human  
CEAmod/42K-promoted B7.1 insertion cassette (Cont.)**

```

3490      3500      3510      3520      3530      3540
AGAAGAAAAT GGTGCTGACT ATGATGTC TG GAGACATGAA TATATGGCCC GAGTACAAGA
TCTTCTTTA CCACGACTGA TACTACAGAC CTCTGTCATT ATATACCGGG CTCATGTTCT
b b HUMAN B7.1 ORF b b >

3550      3560      3570      3580      3590      3600
ACCGGACCAT CTTTGATATC ACTAATAACC TCTCCATTGT GATCCTGGCT CTGCCGCCAT
TGGCCTGGTA GAAACTATAG TGATTTATGG AGAGGTAACA CTAGGACCGA GACGCCGGTA
b b HUMAN B7.1 ORF b b >

3610      3620      3630      3640      3650      3660
CTGACGAGGG CACATACGAG TGTGTTGTT TGAAAGTATGA AAAAGACGCT TTCAAGCGGG
GACTGCTCCC GTGTATGCTC ACACAACAAG ACTTCATACT TTTTCTGCCA AAGTTGCC
b b HUMAN B7.1 ORF b b >

3670      3680      3690      3700      3710      3720
AACACCTGGC TGAAGTGACG TTATCAGTCA AAGCTGACTT CCTCACACCT AGTATATCTG
TTGTGGACCG ACTTCACTGC AATAGTCAGT TTGCACTGAA GGGATGTGGA TCATATAGAC
b b HUMAN B7.1 ORF b b >

3730      3740      3750      3760      3770      3780
ACTTTGAAAT TCCAACCTCT AATATTAGAA GGATAATTG CTCAACCTCT GGAGGTTTTC
TGAAACTTTA AGGTTGAAGA TTATAATCTT CCTATTAAAC GAGTTGGAGA CCTCCAAAAG
b b HUMAN B7.1 ORF b b >

3790      3800      3810      3820      3830      3840
CAGAGCCTCA CCTCTCCTGG TTGGAAAATG GAGAAGAAATT AAATGCCATC AACACAACAG
GTCTCGGAGT GGAGAGGACC AACCTTTAC CTCTTCTTA TTTACGGTAG TTGTGTTGTC
b b HUMAN B7.1 ORF b b >

3850      3860      3870      3880      3890      3900
TTTCCCCAAGA TCCCTGAAACT GAGCTCTATG CTGTTAGCAG CAAACTGGAT TTCAATATGA
AAAGGGTTCT AGGACTTTGA CTCGAGATAC GACAATCGTC GTTTGACCTA AAGTTATACT
b b HUMAN B7.1 ORF b b >

3910      3920      3930      3940      3950      3960
CAACCAACCA CAGCTTCATG TGTCTCATCA AGTATGGACA TTAAAGAGTG AATCAGACCT
GTTGGTTGGT GTCGAAGTAC ACAGAGTAGT TCATACCTGT AAATTCTCAC TTAGTCTGGA
b b HUMAN B7.1 ORF b b >

3970      3980      3990      4000      4010      4020
TCAACTGGAA TACAACCAAG CAAGAGCATT TTCCCTGATAA CCTGCTCCA TCCGGGCCA
AGTTGACCTT ATGTTGGTTC GTTCTCGTAA AAGGACTATT GGACGAGGGT AGGACCCGGT
b b HUMAN B7.1 ORF b b >

4030      4040      4050      4060      4070      4080
TTACCTTAAT CTCAGTAAAT GGAATTTCG TGATATGCTG CCTGACCTAC TGCTTGGCCC
AATGGAATTAA GAGTCATTAA CCTTAAAGC ACTATACGAC GGACTGGATG ACGAACCGGG
b b HUMAN B7.1 ORF b b >

4090      4100      4110      4120      4130      4140
CACGCTGCAG AGAGAGAAGG AGGAATGAGA GATTGAGAAG GGAAAGTGTA CGCCCTGTAT
GTGGCAGTC TCTCTTTCC TCTTACTCT CTAACCTTC CCTTCACAT GCGGGACATA
b b HUMAN B7.1 ORF b b >

4150      4160      4170      4180      4190      4200

```

Figure 4(F)

12/18

**Sequence of the H6-promoted human  
CEAmod/42K-promoted B7.1 insertion cassette (Cont.)**

```

AAGTCGACGA TCCTTTTAT AGCTAATTAG TCACGTACCT TTGAGAGTAC CACTTCAGCT
TTCAGCTGCT AGGAAAAATA TCGATTAATC AGTGCATGGA AACTCTCATG GTGAAGTCGA
-> _____ ALVAC'S C5 <_____
4210      4220      4230      4240      4250      4260
ACCTCTTTG TGTCTCAGAG TAACCTTCTT TAATCAATTG CAAAACAGTA TATGATTTC
TGGAGAAAAAC ACAGAGTC TC ATTGAAAGAA ATTAGTTAAG GTTTTGTCAT ATACTAAAG
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4270      4280      4290      4300      4310      4320
CATTCTTTC AAAGATGTA GTTACATCTG CTCCCTTGTGTT GAAAAGTAGC CTGAGCACTT
GTAAGGAAAG TTTCTACATC AAATGTAGAC GAGGAAACAA CTTTTCATCG GACTCGTGAA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4330      4340      4350      4360      4370      4380
CTTTCTACC ATGAATTACA GCTGGCAAGA TCAATTTC CCAGTTCTGG ACATTTTATT
GAAAAGATGG TACTTAATGT CGACCGTTCT AGTTAAAAG GGTCAAGACC TGTAAAATAA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4390      4400      4410      4420      4430      4440
TTTTTAAGT AGTGTGCTAC ATATTCAT ATTCCAGAT TGACAGCGA TCATTAAGG
AAAAAAATTCA TCACACGATG TATAAAGTTA TAAAGGTCTA ACATGTCGCT AGTAATTTC
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4450      4460      4470      4480      4490      4500
AGTACGTCCC ATGTTATCCA GCAAGTCAGT ATCAGCACCT TTGTTCAATA GAAGTTTAAC
TCATGCAGGG TACAATAGGT CGTTCACTA TAGTCGTGGA AACAAAGTTAT CTTCAAATTG
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4510      4520      4530      4540      4550      4560
CATMTGTTAAA TTTTATTG ATACGGCTAT ATGTAGAGGA GTTAACCGAT CCGTGTGTA
GTAACAATTG AAAAATAAAC TATGCCGATA TACATCTCCCT CAATTGGCTA GCCACAAACT
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4570      4580      4590      4600      4610      4620
AATATCTACA TCCGCCGAAT GAGCCAATAG AAGTTAACCC AAATTAACCT TGTTAAGGTA
TTATAGATGT AGGCCGTTA CTCGGTTATC TTCAATTGG TTAAATTGAA ACAATTCCAT
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4630      4640      4650      4660      4670      4680
ACCTGCCAAA CACAAAGGGAG TAAAGCCTCC GCTGTAAAGA ACATTGTTA CATAGTTATT
TCGACGGTTT GTGTTCTC ATTTGGAGG CGACATTCT GTAAACAAAT GTATCAATAA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4690      4700      4710      4720      4730      4740
CTTCAACAGA TCTTCACTA TTTTGTAGTC GTCTCTAAC ACCGCATCAT GCAGACAAGA
GAAGTTGTCT AGAAAGTGT AAAACATCG CAGAGATTG TGGCTAGTA CGTCTGTCT
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4750      4760      4770      4780      4790      4800
AGTTGTGCAT TCAGTAACTA CAGGTTTACG TCCATACCTC ATCAAGATT TTATAGCTC
TCAACACGTA AGTCATTGAT GTCCAATCG AGGTATGGAG TAGTTCTAAA AATATCGGAG
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >
4810      4820      4830      4840      4850      4860
GGTATTCTTG AACATTACAG CCATTCAAG AGGAGATTGT AGAGTACCAT ATTCCGTGTT

```

Figure 4(G)

13/18

**Sequence of the H6-promoted human  
CEAmad/42K-promoted B7.1 insertion cassette (Cont.)**

```

CCATAAGAAC TTGTAATGTC CGTAAAGTTC TCCCTAAACA TCTCATGGTA TAAGGCACAA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

4870      4880      4890      4900      4910      4920
ACGGTCGAAT CCATGTCCA AAAACCTATT TAGAGATGCA TTGTCATTAT CCATGATAGC
TCCCAGCTTA GGTAAACAGGT TTTGGATAA ATCTCTACGT AACAGTAATA GGTACTATCG
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

4930      4940      4950      4960      4970      4980
CTCACAGACG TATATGTAAG CCATCTTGA TGTTATAATT TGTTGTTTC ACAACCGCT
GAGTGTCTGC ATATACATTC GTTAGAACTT ACATATAAA ACAACAAAAG TTGTTGGCGA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

4990      5000      5010      5020      5030      5040
CGTGAAACAGC TTCTATACCT TTTCATTTT TTCATGATTA ATATAGTTA CGGAATATAA
GCACTTGTGC AAGATATGAA AAAGTAAAAG AAGTACTAAT TATATCAAAT GCCTTATATT
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5050      5060      5070      5080      5090      5100
GTATACAAAA AGTTTATAGT AATCTCATAA TATCTGAAAC ACATACATAA AACATGGAAG
CATATGTTT TCAAATATCA TTAGAGTATT ATAGACTTTC TGTTATGATT TTGTACCTTC
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5110      5120      5130      5140      5150      5160
AATACACGA TGTCTGTCAG ATAATGGCT TTTTATGTC ATAGTTTACA AATTCCGAGT
TTAATGTGCT ACAGCAACTC TATTTACCGA AAAATAACAG TATCAAATGT TTAAGCGCTCA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5170      5180      5190      5200      5210      5220
AATCTTCATC TTTTACGAAT ATTGCAGAACT CTGTTTATC CAACCACTGA TTTTTGTTATA
TTAGAAGTAG AAAATGCTTA TAACGTCTTA GACAAAATAG GTTGGTCACT AAAAACATAT
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5230      5240      5250      5260      5270      5280
ATATAACTGG TATCCTATCT TCCGATAGAA TGCTGTTATT TAACATTTTT GCACCTATTAA
TATATTGACC ATAGGATAGA AGGCTATCTT ACGACAATAA ATTGTAAAAA CGTGGATAAT
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5290      5300      5310      5320      5330      5340
AGTTACATCT GTCAAATCCA TCTTTCCAAC TGACTTTATG TAACGATGCC AAATAGCATT
TCAATGTAGA CAGTTTAGGT AGAAAGGGTG ACTGAAATAC ATTGCTACGC TTTATCGTAA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5350      5360      5370      5380      5390      5400
TATCACTATG TCGTACCCAA TTATCATGAC AAGATCTCT TAATACGTA ATCTTATTAT
ATAGTCATAC AGCATGGGT AATAGTACTG TTCTAAGAGA ATTATGCTAT TAGAATAATA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5410      5420      5430      5440      5450      5460
CTCTTGCATA TTCTGTAATG TAATTTAAA GAGTATACGA TAACAGTATA GATATACACG
GAGAACGTAT AAGCATTATC ATTAACATT CTCATATGCT ATTGTCTATAT CTATATGTCC
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5470      5480      5490      5500      5510      5520
TGATATAAAAT ATTTAACCCC ATTCTGAGT AAAATAATTG CGATATTACA TTTCTTTTA
ACTATATTAA TAAATTGGGG TAAGGACTCA TTTTATTAAT GCTATAATGT AAAGGAAAAT
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

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Figure 4(H)

14/18

**Sequence of the H6-promoted human  
CEAmid/42K-promoted B7.1 insertion cassette (Cont.)**

```

5530      5540      5550      5560      5570      5580
TTATTTTAT GTTTAGTTA TTTGTTAGGT TATAACAAAAA TTATGTTAT TTGTGTATAT
AATAAAAATA CAAATCAAT AAACAATCCA ATATGTTTT AATACAATA AACACATATA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5590      5600      5610      5620      5630      5640
TTAAAGCGTC GTTAAGAATA AGCTTAGTTA ACATATTATC GCTTAGGTTT TGTAGTATTT
AATTCGCAG CAATTCTTAT TCGAATCAAT TGTATAATAG CGAATCCAAA ACATCATAAA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5650      5660      5670      5680      5690      5700
GAATCCTTTC TTAAATGGA TTATTTTCC AATGCATATT TATAGCTTCA TCCAAAGTAT
CTTAGGAAAG AAATTTACCT AATAAAAAGG TTACGTATAA ATATCGAAGT AGGTTTCATA
d ALVAC'S C5 LOCUS RIGHT FLANKING ARM d >

5710      5720      5730      5740      5750      5760
AACATTTAAC ATTCAAGAATT GCGCCCGAA TTCATTGCGT AATCATGGTC ATAGCTGTTT
TTGTAATATG TAAGTCTTAA CGCCGGCGTT AAGTTAAGCA TTAGTACCAAG TATCGACAAA
d ALVAC'S C5 LOCUS d >

5770      5780      5790      5800      5810      5820
CCTGTGTGAA ATTGTTATCC GCTCACAAATT CCACACAAACA TACGACCCCCG AAGCATAARG
GGACACACTT TAACAATAGG CGAGTGTAA GGTGTGTGT ATGCTCGGCC TTCGTATTTC

5830      5840      5850      5860      5870      5880
TGTAACGCTT GGGGTGCCTA ATGAGTGAGC TAACTCACAT TAATTGCGTT GCGCTCACTG
ACATTTCGGA CCCCACGGAT TACTCACTCG ATTGAGTGTAA ATTAACCCAA CGCGAGTGAC

5890      5900      5910      5920      5930      5940
CCCGCTTTCAGTCGGGAAA CCTGTCGGTC CAGCTGCATT AATGAATCGG CCAACCGCG
GGGCGAAAGG TCAGCCCTTT GGACAGCACG GTCGACGTAA TTACTTAGCC GGTTGCGCGC

5950      5960      5970      5980      5990      6000
GGGAGAGGCG GTTTCGCTAT TGGGCGCTCT TCCGCTTCCT CGCTCACTGA CTCGCTCGCC
CCCTCTCCGC CAAACGCATA ACCCGCGAGA AGGCGAAGGA GCGAGTGACT GAGCGACCG

6010      6020      6030      6040      6050      6060
TCGGTCGTTTC GGCTGCGGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAAT ACGGTTATCC
AGCCAGCAAG CGCACGCCGC TCGCCATAGT CGAGTGAGTT TCCGCCATTA TGCCAATAGG

6070      6080      6090      6100      6110      6120
ACAGAAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG
TGTCTTAGTC CCCTATTGCG TCCTTCTTG TACACTCGTT TTCCGGTCGT TTCCGGTCC

6130      6140      6150      6160      6170      6180
AACCCTAAAAA AGGCCGGCGTT GCTGGCGTTT TTCCCATAGGC TCCGCCCCCCC TGACGAGCAT
TTGGCATTTC TCCGGCGCAA CGACCGCAA AAGGTATCCG AGGCGGGGGG ACTGCTCGTA

6190      6200      6210      6220      6230      6240
CACAAAAATC GACGCTCAAG TCAGAGGTGG CGAAACCGA CAGGACTATA AAGATACCAAG
GTGTTTTAG CTGCGAGTTC AGTCTCCACC GCTTTGGGCT GTCTGTATAT TTCTPATGGTC

6250      6260      6270      6280      6290      6300
GCGTTTCCCC CTGGAAAGCTC CCTCGTGCCTC TCTCCCTGTT CGACCCCTGCC GCTTACCGGA
CGCAAAAGGGG GACCTTCGAG GGAGCACCG AGAGGGACAAG GCTGGGACGG CGAATGGCT

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Figure 4(I)

**Sequence of the H6-promoted human  
CEAmid/42K-promoted B7.1 insertion cassette (Cont.)**

|   |      |      |      |      |      |
|---|------|------|------|------|------|
| 6310  | 6320 | 6330 | 6340 | 6350 | 6360 |
| 'TACCTGTCCG CCTTTCTCCC TTGGGAAGC GTGGCGCTTC CTCATAGCTC ACGCTGTAGG   |      |      |      |      |      |
| ATGGACAGGC GGAAAGAGGG AAGGCCCTCG CACCGCGAAA GAGTATCGAG TGCGACATCC   |      |      |      |      |      |
| 6370  | 6380 | 6390 | 6400 | 6410 | 6420 |
| TATCTCAGTT CGGTGTAGGT CGTTCGCTCC AAGCTGGCT GTGTGCACGA ACCCCCCGTT    |      |      |      |      |      |
| ATAGAGTCAA GCCACATCCA GCAAGCGAGG TTCGACCCGA CACACGTGCT TGGGGGGCAA   |      |      |      |      |      |
| 6430  | 6440 | 6450 | 6460 | 6470 | 6480 |
| CAGCCCGACC GCTGCGCTT ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC    |      |      |      |      |      |
| GTCCGGCTGG CGACCGGGAA TAGGCCATTG ATAGCAGAAC TCAGGGTTGGG CCATTCTGTG  |      |      |      |      |      |
| 6490  | 6500 | 6510 | 6520 | 6530 | 6540 |
| GACTTATCGC CACTGGCAGC AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC   |      |      |      |      |      |
| CTGAATAGCG GTGACCGCTCG TCGGTGACCA TTGTCTTAAT CGTCTCGCTC CATAACATCCG |      |      |      |      |      |
| 6550  | 6560 | 6570 | 6580 | 6590 | 6600 |
| GGTGCTACAG AGTTCTTGAA GTGGTGGCT AACTACGGCT ACACTAGAAG GACAGTATTT    |      |      |      |      |      |
| CCACGATGTC TCAAGAACTT CACCAACCGGA TTGATGCCGA TGTGATCTTC CTGTCAAAA   |      |      |      |      |      |
| 6610  | 6620 | 6630 | 6640 | 6650 | 6660 |
| GGTATCTGCG CTCTGCTGAA GCCAGTTAACC TTGGAAAAAA GAGTTGGTAG CTCTTGATCC  |      |      |      |      |      |
| CCATAGACCC GAGACGACTT CGGTCAATGG AAGCCTTTTT CTCAACCATC GAGAACTAGG   |      |      |      |      |      |
| 6670  | 6680 | 6690 | 6700 | 6710 | 6720 |
| GGCAAAACAAA CCACCGCTGG TAGCGGTGGT TTTTTTGTGTT GCAAGCAGCA GATTACGCGC |      |      |      |      |      |
| CCGTTTGTGTT GGTGGCGACC ATCGCCACCA AAAAAACAAA CGTTCGTCGT CTAATGCGCG  |      |      |      |      |      |
| 6730  | 6740 | 6750 | 6760 | 6770 | 6780 |
| AGAAAAAAAG GATCTCAAGA AGATCCCTTG ATCTTTCTA CGGGGTCTGA CGCTCAGTGG    |      |      |      |      |      |
| TCTTTTTTCTC CTAGAGTTCT TCTAGGAAAC TAGAAAAGAT GCCCCAGACT CGAGTCACC   |      |      |      |      |      |
| 6790  | 6800 | 6810 | 6820 | 6830 | 6840 |
| AACGAAACT CACGTTAAGG GATTTTGGTC ATGAGATTAT CAAAAGGAT CTTCACCTAG     |      |      |      |      |      |
| TTGTTTTGAA GTGCAATTCC CTAAAACCAAG TACTCTAATA GTTTTCTCTA GARGTGGATC  |      |      |      |      |      |
| 6850  | 6860 | 6870 | 6880 | 6890 | 6900 |
| ATCCTTTAA ATTAAAAATG AAGTTTAAA TCAACTAAA GTATATATGA GTAAACTTGG      |      |      |      |      |      |
| TAGGAAAATT TAATTTTAC TTCAAAATT AGTTAGATT CATATATACT CATTGAAACC      |      |      |      |      |      |
| 6910  | 6920 | 6930 | 6940 | 6950 | 6960 |
| TCTGACAGTT ACCAATGCTT AATCAGTGAG GCACCTATCT CAGCGATCTG TCTATTTCTG   |      |      |      |      |      |
| AGACTGTCAA TGGTACGAA TTAGTCACTC CGTGGATAGA GTCGCTAGAC AGATAAAAGCA   |      |      |      |      |      |
| 6970  | 6980 | 6990 | 7000 | 7010 | 7020 |
| TCATCCATAG TTGCTGACT CCCCGCTGTG TAGATAACTA CGATACGGGA GGGCTTACCA    |      |      |      |      |      |
| AGTAGGTATC AACGGACTGA GGGCAGCAC ATCTATTGAT GCTATGCCCT CCCGAATGGT    |      |      |      |      |      |
| 7030  | 7040 | 7050 | 7060 | 7070 | 7080 |
| TCTGGCCCCA GTGCTGCAAT GATACCGCGA GACCCACGCT CACCGGCTCC AGATTTATCA   |      |      |      |      |      |
| AGACCGGGGT CACGACGTTA CTATGGCGCT CTGGGTGCGA GTGGCCGAGG TCTAAATAGT   |      |      |      |      |      |
| 7090  | 7100 | 7110 | 7120 | 7130 | 7140 |
| GCAATAAACCG AGCCAGCGG AAGGGCCAG CGCAGAAGTG GTCTGCAAC TTTATCCGCC     |      |      |      |      |      |
| CGTTATTTGG TCGGTGGCCC TTCCCGCTC CGCTCTTCAC CAGGACGTTG AAATAGGGGG    |      |      |      |      |      |
| 7150  | 7160 | 7170 | 7180 | 7190 | 7200 |

Figure 4(J)

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**Sequence of the H6-promoted human  
CEAmid/42K-promoted B7.1 insertion cassette (Cont.)**

```

TCCATCCAGT CTATTAATTG TTGCCGGAA GCTAGAGTAA GTAGTTGCC AGTTAATAGT
AGGTAGGTCA GATAATTAAC AACGGCCCTT CGATTCATT CATCAAGCGG TCAATTATCA

    7210      7220      7230      7240      7250      7260
TTGCGCAACG TTGTTGCCAT TGCTACAGGC ATCGTGGTGT CACGCTCGTC GTTGTGATAG
AACGCCTGTC AACACAGGTAA ACGATGTCGG TAGCACCAACA GTGCGAGCAG CAAACCATAAC

    7270      7280      7290      7300      7310      7320
GCTTCATTCA GCTCCGGTTC CCAACGATCA AGGCGACTTA CATGATCCCC CATGTTGTGCG
CGAAGTAAAGT CGAGGCCAAG GGTTGCTAGT TCCGCTCAAT GTACTAGGGG GTACAAACACG

    7330      7340      7350      7360      7370      7380
AAAAAAAGCGG TTAGCTCCTT CGGTCCCTCG ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG
TTTTTCGCC AACAGGAA GCCAGGAGG TAGCAACAGT CTTCATCAA CGGGCGTCAC

    7390      7400      7410      7420      7430      7440
TTATCACTCA TGGTTATGGC AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA
AAATAGTGAGT ACCAACACCG TCGTGACGTA TAAAGAGAAT GACAGTACGG TAGGCATTCT

    7450      7460      7470      7480      7490      7500
TGGTTTCTG TGACTGGTGA GTACTCAACC AAGTCATTCT GAGAATAGTG TATGCGGGCA
ACGAAAAGAC ACTGACCACT CATGAGTTGG TTCAGTAAGA CTCTTATCAC ATACGCCGCT

    7510      7520      7530      7540      7550      7560
CCGAGTTAGCT CTTGCCCGGC GTCAATAACGG GATAATACCG CGCCACATAG CAGAACTTTA
GGCTCAACGA GAACGGGCCG CAGTTATGCC CTATTATGGC GCGGTGTATC GTCTTGAAAT

    7570      7580      7590      7600      7610      7620
AAAGTGCTCA TCATTGGAAA ACAGTTCTCG GGGCGAAAAC TCTCAAGGAT CTTACCGCTG
TTTCACGAGT AGTAACCTTT TGCAAGAAC CCCGCTTTG AGAGTTCCCTA GAATGGCGAC

    7630      7640      7650      7660      7670      7680
TTGAGATCCA GTTCGATGTA ACCCACTCGT GCACCCAACT GATCTTCAGC ATCTTTACT
AACTCTAGGT CAAGCTACAT TGGGTGAGCA CGTGGGTTGA CTAGAAGTCG TAGAAAATGA

    7690      7700      7710      7720      7730      7740
TTCACCAGCG TTCTGGGTG AGCAAAACA GGAAGGCAAA ATGCCGAAA AAAGGGAATA
AAGTGGTCGC AAAGACCCAC TCGTTTTGT CCTTCGTTT TACGGCGTTT TTTCCCTTAT

    7750      7760      7770      7780      7790      7800
AGGGCGACAC GGAAATGTG AATACTCATA CTCTCCCTT TCAATATTA TTGAAGCATT
TCCCCTGTG CTTTACAAC TTATGAGTAT GAGAAGGAAA AAGTTATAAT AACTTCGTAA

    7810      7820      7830      7840      7850      7860
TATCAGGGTT ATAGTCTCAT GAGCGGATAC ATATTGAAAT GTATTTAGAA AAATAACAA
ATAGTCCCAA TAACAGAGTA CTCGCCTATG TATAAACTTA CATAATCTT TTTATTTGTT

    7870      7880      7890      7900      7910      7920
ATAGGGGTT CGCGCACATT TCCCCAAAAA GTGCCACCTG ACGTCTAAGA AACCAATTATT
TATCCCCAAG GCGCGTGTAA AGGGCTTTT CACGGTGGAC TGCAGATTCT TTGGTAATAA

    7930      7940      7950
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TAGTACTGTA ATTGGATATT TTTATCCGCA TAGTGCTC

```

Figure 4(K)

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### Immunoprecipitation Analysis



Lane 1: Uninfected HeLa cells

Lane 2: HeLa cells infected with ALVAC(2) parental virus (vCP1468)

Lane 3: HeLa cells infected with ALVAC-CEA/B7.1 (vCP307)

Lane 4: HeLa cells infected with ALVAC-B7.1 (vCP1334)

Lane 5: HeLa cells infected with ALVAC-CEAm/B7.1 (vCP1585)

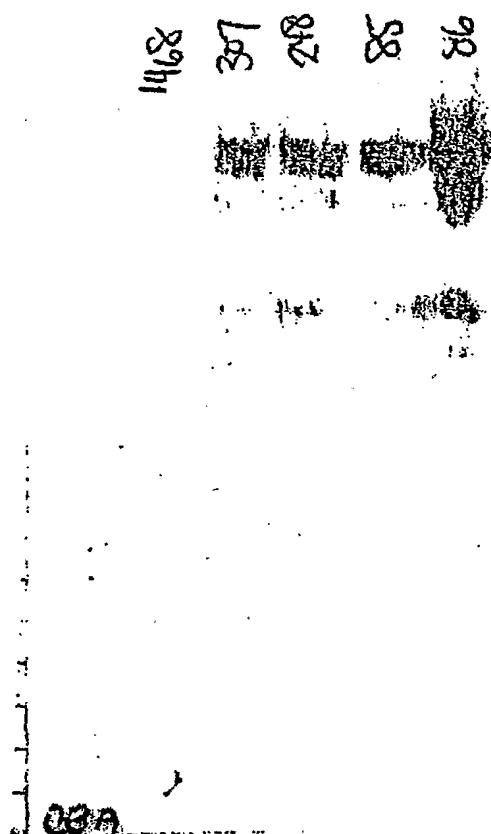
Lane 6: HeLa cells infected with ALVAC(2)-CEAm/B7.1 (vCP1586)

Molecular Weight Markers: 200, 98.6, 68, 43, 29, 18, 14 kDa

**Figure 5**

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### Western Blot Analysis



- Lane 1: HeLa cells infected with ALVAC(2) parental virus (vCP1468)  
Lane 2: HeLa cells infected with ALVAC-CEA/B7.1 (vCP307)  
Lane 3: HeLa cells infected with ALVAC-CEA (vCP248)  
Lane 4: HeLa cells infected with ALVAC-CEAm/B7.1 (vCP1585)  
Lane 6: HeLa cells infected with ALVAC(2)-CEAm/B7.1 (vCP1586)

**Figure 6**

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SEQUENCE LISTING

<110> AVENTIS PASTEUR LIMITED  
THERION BIOLOGICS  
NATIONAL CANCER INSTITUTE  
Berinstein, Neil  
Tartaglia, James  
Tine, John, A.  
Panicali, Dennis L.  
Gritz, Linda  
Schlom, Jeffrey

<120> MODIFIED CEA AND USES THEREOF

<130> 1038-1144 LAB

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His Leu Phe Gly Tyr Ser Trp Tyr Lys Gly Glu Arg Val Asp Gly Asn Arg Gln Ile Ile  
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-2-

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-3-

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| atatacccca | atgcataccct | gctgatccag  | aacatcatcc | agaatgacac | aggattctac | 360 |
| accctacacg | tcataaaagtc | agatcttgt   | aatgaagaag | caactggcca | gttccgggta | 420 |
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